REMARKS

I. Status of the Application

The Examiner states in paragraph 2 at page 2 in the instant Office Action that claims 1-8, 10-15, and 37-*52* are pending. Applicants note, however, that new claims 40-*56* were entered in the Response dated January 29, 2002. Accordingly, it is presumed that Claims 1-8, 10-15 and 37-56 are presently pending in the application.

Claims 1-8, 10-15, 37-39, and 40-56 stand rejected under 35 U.S.C. § 102(e), or alternatively under 35 U.S.C. § 103(a) over US 5,650,489 ("Lam"). Claims 1-8, 10-15, 37-39, and 40-56 are rejected under § 103(a) over Lam in view of US 5,679,773 ("Holmes"). Claims 40-48, 50, and 52-56 stand rejected under 35 U.S.C. § 112, first paragraph.

Applicants thank the Examiner for withdrawing the obviousness-type double patenting rejection over US Patent Nos. 5,843,655 and 6,238,862.

All pending independent claims (i.e., claims 1, 10, 40, and 50) have been amended to clarify the subject matter defined by the claims. Specifically, Applicants have amended each of the pending independent claims to recite that the diverse polymers are spatially defined on the solid substrate on which the preselected array is synthesized. Applicants respectfully submit that no new matter is being presented in the amendments above. Support for the amendments above can be found throughout the specification, for example, at page 15, lines 26-29, which states:

> A "preselected array of polymers" is a spatially defined pattern of polymers on a solid support which is designed before being constructed (*i.e.*, the arrangement of polymers on solid substrate during synthesis is deliberate, and not random).

In particular, Applicants have amended the claims above to make it clearer that the same solid support: (1) serves as a base to synthesize a preselected array thereon; **and** (2) serves as a base to support spatially defined polymers thereon. In contrast, the solid support in Lam: (1)

serves as a base to synthesize one polymer thereon; and (2) **cannot** serve to spatially define the polymer thereon. Lam requires a support separate from the "synthesizing support" (i.e., a 96-well microtiter plate) to spatially define the polymers formed, as discussed more fully below. Because of these and other remarks, which follow, Applicants sincerely believe that all of the pending claims are patentable and an indication to that effect is respectfully requested at this time.

II. Claims 1-8, 10-15, 37-39 and 40-56 Are Patentable Over Lam

Claims 1-8, 10-15, 37-39, and 40-56 stand rejected under § 102(e), or alternatively under § 103(a) over Lam for the reasons set forth in a previous Office Action mailed on October 5, 2001 (Paper No. 38). Applicants respectfully traverse the rejection in view of the amendments and remarks presented here.

Applicants submit that Claim 40 (the broadest pending independent claim) is patentable over Lam because Lam does not disclose or otherwise teach or suggest at least the claim elements discussed immediately below. Claim 40, as amended, is reproduced below:

> 40. (Currently Amended) A method of monitoring polymer array synthesis on a solid substrate comprising:
>
> (i) synthesizing a preselected array of diverse polymers connected to cleavable linkers on a solid substrate, whereby the diverse polymers occupy different regions of the solid substrate and are spatially defined on the solid substrate in which the preselected array is synthesized;
>
> (ii) cleaving diverse polymers from the solid substrate by cleaving the cleavable linkers, thereby creating a mixture of diverse unbound polymers; and
>
> (iii) measuring presence of diverse unbound polymers as an indicator of the efficiency of the synthesizing step.

A. Lam does not disclose "polymers . . . spatially defined on the solid substrate in which the preselected array is synthesized" because the support Lam uses for polymer synthesis is not capable of serving as a base to support spatially defined diverse polymers.

First, it is obvious from the disclosure of Lam at column 22, lines 34-38 that the polymers are not spatially defined on the same substrate used for polymer synthesis:

> A library of sequentially cleavable bio-oligomers is prepared and distributed in wells of microtiter plates such that **each well contains** more than about 50, and more preferably from about 50 to about 250, **beads** per well. (Emphasis added).

If the support used for synthesizing the polymers in Lam *were* capable of serving as a base to support spatially defined polymers, then the above-cited step of distributing beads containing bio-oligomers in the wells of a microtiter plate would not be necessary. It is apparent then that Lam places the beads in the microtiter plate to simply contain the solid support used for synthesis (i.e., the beads).

Second, the solid support Lam uses for polymer synthesis cannot serve as a base to support spatially defined polymers since the support Lam uses for polymer synthesis cannot support more than one polymer, as discussed immediately below (see "one bead-one bio-oligomer").

Thus, Lam fails to disclose "polymers . . . spatially defined on the solid substrate in which the preselected array is synthesized."

B. Lam does not disclose "a preselected array of diverse polymers" because Lam teaches split bead synthesis, which is incapable of forming an array of diverse polymers on a solid support in which the preselected array is synthesized.

Applicants define at page 15, lines 26-29, a preselected array of polymers as ". . . a spatially defined pattern of **polymers** on **a** solid support which is designed before being

constructed. . ." (Emphasis added). Thus, a preselected array is characterized by having ***more than one polymer on a single solid support.***

Lam teaches throughout its disclosure split bead synthesis, which is fundamentally different from the array synthesis disclosed by Applicants. One fundamental difference is that split bead synthesis is limited to there being only one polymer per solid support (i.e., the bead). Lam acknowledges this limitation of split bead synthesis at column 7, lines 47-53:

> In the method of the invention, at least two aliquots of solid phase support are provided wherein the number of solid phase supports in the aliquots preferably correspond to at least the number of bio-oligomers to be synthesized. This permits the creation of a library in which **each solid phase support contains a single bio-oligomer species, i.e., one bead-one bio-oligomer**. (Emphasis added).

It is thus apparent then the method of Lam cannot have more than one polymer on a single solid synthesis support. Even if one were to argue that the solid support disclosed by Lam does have more than one (albeit the same) polymer on the solid support, there certainly cannot be ***diverse*** polymers on the same single support. This is because of the inherent limitation in split bead synthesis described above.

Those of skill in the art appreciate this distinction between split bead libraries and array synthesis. For example, the attached article published as a special feature in *Chemical & Engineering News* in 1996 discusses the fundamental differences between array synthesis (of Applicants) and split bead libraries (disclosed by Lam). See, for example, "Creating Libraries" at page 2 of Attachment A. In addition, a recent article recently published in the *Journal of Combinatorial Chemistry* also confirms differences between arrays defined by the present claims and split (bead) libraries taught by Lam. See "Introduction" in Attachment B.

Thus, Lam does not disclose a preselected array of diverse polymers because the support Lam uses for split bead polymer synthesis cannot support an array of (more than one) diverse

polymer. Since Lam does not disclose a preselected array of diverse polymers, it naturally follows that Lam also does not disclose or otherwise teach or suggest diverse polymers occupying different regions of the substrate, as defined by Claim 40.

C. Lam's disclosure of a predetermined sequence is not a teaching of a preselected array because a predetermined sequence relates to the order of the monomers in the individual polymer synthesized whereas a preselected array relates to the arrangement of the polymers themselves on the solid substrate.

Examiner Ponnaluri asserts on page 9, paragraph 14 of the Office Action dated April 23, 2002, "Applicants arguments [Ed: regarding the claim element "preselected array"] have been fully considered and are not persuasive, because Lam et al teach the method can be used for synthesis of random library as well as for the synthesis of peptide library that comprise a predetermined sequence (see column 10, lines 57-59)."

Applicants submit that Lam's "peptide library that comprises a predetermined sequence" is drastically different from a preselected array. Lam is merely referring to a library of peptides having known sequences because the amino acids are added to the polymer chain in a predetermined order. In contrast, Applicants' preselected arrays refer to a spatially defined pattern of polymers on a solid support, as evidenced by Applicants' definition of "preselected array" above. It is one thing to form a peptide with a known sequence, and it is an entirely different thing to form an array of polymers. In essence, Examiner Ponnaluri is comparing apples and oranges and appears to be confusing two fundamentally distinct concepts. Accordingly, Lam's library of peptides having predetermined sequences does not teach or suggest Applicants' preselected arrays. To the extent Applicants are misreading the basis for the Examiner's rejection, Applicants respectfully request that the Examiner elaborate on the meaning

of "peptide library that comprise a predetermined sequence" and demonstrate where a "preselected array" as defined by Applicants is disclosed in Lam.

Since Lam fails to disclose or otherwise teach or suggest all of the limitations of claim 40 as discussed above, claims 41-49 are patentable over Lam by virtue of their direct or indirect dependency from claim 40. Similarly, all other pending independent claims (i.e., claims 1, 10 and 50) are patentable over Lam for at least the same reasons presented above since each of these independent claims also define each of the claim elements discussed above. Accordingly, claims 2-8, 11-15, 37-39, and 51-56 are patentable over Lam by virtue of their direct or indirect dependency.

III. Claims 1-8, 10-15, 37-39 and 40-56 Are Patentable Over Lam in view of Holmes

Claims 1-8, 10-15, 37-39, and 40-56 are rejected under § 103(a) over Lam in view of Holmes for the reasons set forth in a previous Office Action mailed on October 5, 2001 (Paper No. 38). Applicants respectfully traverse the rejection for the reasons discussed below.

A. The teachings of Lam and Holmes cannot be combined to render the present claims obvious because the combination of the references is improper.

Applicants believe that it is improper to combine Lam with Holmes to cure the deficiency of Lam (i.e., for the teaching of a preselected array of diverse polymers on a substrate, whereby the diverse polymers are spatially defined on the solid substrate in which the preselected array is synthesized) because Lam teaches away from spatially defined synthesis.

Lam dismisses "light-directed spatially addressable parallel chemical synthesis" as being "limited" at column 3, lines 45-52. Examiner Ponnaluri is respectfully reminded that a prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead

away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). Certainly, a person of ordinary skill reading this disclosure of Lam would not be led to make the cited combination because of the cited limitations of array synthesis, which Lam discusses in its Background section. Further, a person of ordinary skill in the art seeking to do array synthesis would not consider Lam, which teaches split bead synthesis, because of the inherent limitations of split bead synthesis discussed above (e.g., "one polymer per bead").

In reply to Applicants' previous responses that the combination of Lam and Holmes is improper, Examiner Ponnaluri asserted on page 9, paragraph 11 of Paper No. 38 that Applicants' assertions are unpersuasive because "a biooligomer library may be composed of a predetermined limited number of subunits" and that "this method [of Lam] may be used for synthesis of random peptide libraries as well as for the synthesis of a peptide library that comprised of predetermined sequences." Again, Applicants believe that Examiner Ponnaluri is confusing synthesizing a peptide having a known sequence (which is what Lam discloses) versus synthesizing a preselected array of diverse polymers that are spatially defined on the substrate in which the preselected array is synthesized (as claimed by Applicants and not taught by Lam).

Applicants believe the issue the Examiner should focus on is whether Lam is synthesizing an array of diverse polymers on a substrate, whereby the diverse polymers are spatially defined on the same solid substrate used for synthesis. Applicants faithfully submit Lam is not. As discussed above, it is inconsequential that Lam teaches a predetermined sequence.

B. The teachings of Lam and Holmes cannot be combined to render the pending claims obvious because the combination would change the principle of operation of Lam, which is impermissible.

That is, the combination would require Lam to adopt array synthesis over split bead synthesis to synthesize polymers. Of course, such a modification would fundamentally change the principle of operation of Lam because, as discussed above, split bead synthesis (taught by Lam) is fundamentally different from array synthesis (taught by Holmes).

Examiner Ponnaluri is respectfully reminded that if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959). In *In re Ratti*, the CCPA reversed the rejection holding the "suggested combination of references would require a substantial reconstruction and redesign of the elements shown in [the primary reference] as well as a change in the basic principle under which the [primary reference] construction was designed to operate." 270 F.2d at 813, 123 USPQ at 352.

Accordingly, the combination of references in the present case would change the fundamental procedure in which Lam performs combinatorial chemistry (i.e., from split bead synthesis to array synthesis) and therefore is an improper combination.

Therefore, for at least the reasons discussed above, it is not obvious to monitor the synthesis of polymer arrays synthesized on a support as taught by Holmes using a method of analysis as taught by Lam, as suggested by the Examiner on page 6, paragraph 9 of Paper No. 38. Accordingly, Applicants respectfully request removal of the present rejection at this time.

IV. Claims 40-48, 50, and 52-56 Are Supported by the Specification

Claims 40-48, 50, and 52-56 stand rejected under § 112 for the reasons stated in the Office Action dated 4/23/02 (i.e., because the specification does not adequately support the term "polymer" as presently claimed because the specification is allegedly directed to peptide and nucleotide libraries, which is said to not provide adequate representation of the claimed method of preparing the genus (i.e., polymer arrays). Applicants respectfully traverse this rejection for the reasons discussed below.

A. The written description indicates that Applicants were in possession of arrays of compounds other than peptides and oligonucleotides given the high level of skill in the polymer synthesis art.

Applicants respectfully remind Examiner Ponnaluri, "[T]he 'essential goal' of the description of the invention requirement is to clearly convey the information that an applicant has invented the subject matter which is claimed." *In re Barker*, 559 F.2d 588, 592 n.4, 194 USPQ 470, 473 n.4 (CCPA 1977). In determining whether the written description requirement is satisfied, patents and printed publications in the art should be relied upon to determine the maturity and level of skill in the art. Undoubtedly, the level of a person of ordinary skill in the art of polymer synthesis art is high given the complexity of the art. Accordingly, Applicants need not provide an example for *each and every* conceivable polymer that can be made via Applicants' disclosure.

B. The written description indicates that Applicants were in possession of arrays of polymers other than peptides and oligonucleotides.

For instance, Cho et al. disclosed polycarbamates in 1993. See Attachment C. Cho et al. (1993). An Unnatural Biopolymer. *Science* 261:1303-1305. In addition, Briceno et al.

disclosed in October 1995 non-biological materials discovered via combinatorial methods. See Attachment D. Briceno et al. (1995). A Class of Cobalt Oxide Magnetoresistance Materials Discovered with Combinatorial Synthesis. *Science* 270:273-275. Examples of arrays of non-biological polymers were known by those of skill in the art at the time of application. Schultz et al. disclosed the synthesis of an array of different organic polymers at Example B in US Patent No. 5,985,356 filed 10/18/1994. See Attachment E. The '356 patent is directed towards non-biological organic polymers and explicitly excludes α-amino acids and nucleotides at column 7, lines 41-56.

Examiner Ponnaluri asserts at paragraph 9 of the present Office Action (Paper No. 46), "at the time the invention was made it was not well known in the art to make polymer array synthesis other than peptide or oligonucleotides array." Clearly, in view of the evidence presented above, the Examiner's assertion is without merit.

C. It is evident that Applicants were in possession of arrays of compounds other than peptides and oligonucleotides given that Applicants provided adequate written description to demonstrate to a person of ordinary skill in the art that they were in possession of the claimed subject matter at the time of filing.

For example, Applicants disclose how to couple nucleic acids to a solid support. A person of ordinary skill in the art in possession of Applicants' disclosure and the knowledge generally available could readily link cleavable molecules other than amino acids and nucleic acids to a solid support. For example, the *Science* article published in 1993 (Attachment C) describes at pp. 1303-1304 how to couple a carbamate to a resin support and how to cleave a polymer from the resin (e.g., by subjecting the resin support having the polymer attached to trifluroacetic acid). Between the knowledge available to those of skill in the art at the time of application and Applicants' teaching, e.g., at p. 20 of the application that describes how to couple

a nucleic acid monomer to a solid substrate, the present application adequately encompasses and describes not only how to couple monomers of biologically related polymers to a solid substrate but also, how to couple monomers of *non*-biologically related polymers to a solid substrate.

Further, Applicants' disclosure of labeling the polymers is sufficient to convey to skilled artisans that they were in possession of the claimed subject matter at the time of filing. Applicants describe for at least 14 pages between pages 25 and 39 of the present application how to label polymers. For example, at page 25, lines 22-27, Applicants state:

> In preferred embodiments, labels of the present invention have the structure A-B, where A is a detectable moiety, and B is a 'linking' or 'bridging' group which comprises one or more functional regions which allow the detectable moiety to be incorporated into a polymer, or attached to one end of the polymer, using chemistry similar to that used to connect monomers into the polymer.

In addition, examples of labeling polymers synthesized on a solid support were known to those of skill in the art at the time of application. For example, Nestler et al. disclosed in 1994 a general method for molecular tagging of encoded libraries. See Attachment F. Thus, a person of ordinary skill in the art at the time of application would be able to ascertain that Applicants possessed the claimed subject matter at the time of application given the benefit of Applicants' disclosure.

Examiner Ponnaluri asserts, "the specification does not give guidance on how to link the monomers (other than amino acids and nucleic acids) of the polymers to the support such that they are cleavable or how to label the polymers..." See paragraph 9 of the present Office Action (Paper No. 46). It is apparent from the above discussion that the Examiner's assertions are without merit.

Since Examiner Ponnaluri has not apparently satisfied the initial burden of presenting evidence or reasoning (with support) to explain why persons skilled in the art would not

recognize in the original disclosure a description of the inventions defined by the claims, Applicants respectfully request removal of the present rejection at this time. See *In re Wertheim*, 541 F.2d 257, 191 USPQ 90, 97 (CCPA 1976). ("[T]he PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.").

V. Claims 40-48, 50, and 52-56 Are Enabled

Claims 40-48, 50, and 52-56 stand rejected under § 112 as not enabling arrays of polymers other than nucleotides, peptides, and peptide nucleic acids for the reasons stated in the Office Action dated 4/23/02. Applicants respectfully traverse this rejection in view of the following discussion.

A. Synthesizing arrays of compounds other than peptides and oligonucleotides would not require undue experimentation given the state of the art at the time of filing the present application and the benefit of Applicants' disclosure.

Applicants appreciate Examiner Ponnaluri's thoroughness in the Office Action dated 4/23/02 beginning at page 5 in which each factor relating to the test for undue experimentation was analyzed. Examiner Ponnaluri was incorrect, however, in asserting that "The preparation of arrays of diverse arrays of polymers . . . does not appear to be within the scope of reasonable experimentation."

With reference to paragraph 1 at page 6 in the Office Action dated 4/23/02, Applicants have established above that the present disclosure is adequate to demonstrate to a person of ordinary skill in the art that they were in possession of the claimed subject matter (synthesizing a preselected array of diverse polymers) at the time of filing. As such, a person of ordinary skill in

the art would be enabled to make such arrays of polymers, wherein the term "polymers" includes polymers other than peptides and oligonucleotides.

With reference to paragraph 2 at page 6 in the same Office Action, Applicants note that they need not provide working examples for each and every conceivable polymer that can be made via Applicants' disclosure. Further, as indicated immediately above, the present disclosure does enable a person of ordinary skill in the art to make arrays of polymers other than peptides and oligonucleotides given the working examples that Applicants *did* provide (see Examples 1-2, pages 41-50). Accordingly, Examiner Ponnaluri's assertion at page 5 in the present Office Action that Applicants' previous arguments are not persuasive because "Applicants have shown how polymers other than the peptides and oligonucleotides are synthesized on a support and monitored for efficiency" is without merit.

With reference to paragraph 5 at page 6 in the same Office Action, Applicants have established above through evidence that arrays of polymers other than peptides and oligonucleotides were known at the time of filing. As such, a person of ordinary skill in the art would be enabled to apply the present methods to making arrays of such non-biological polymers given the benefit of Applicants' disclosure. Accordingly, Examiner Ponnaluri assertion at page 5 in the present Office Action that Applicants' previous arguments are not persuasive because "at the time the invention was made the methods of synthesis of polymers other than peptides and oligonucleotides on an array by attaching step by step individual monomers to a solid support are not known" is unfounded.

Applicants respectfully remind Examiner Ponnaluri that the test of enablement is, "whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." *United*

States v. Telectronics, Inc., 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). Further, a patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). Certainly, Applicants need not provide examples of the synthesis of arrays of each and every possible polymer that can be synthesized using the present methods.

B. A person of ordinary skill in the art could readily make and use such arrays of polymers other than nucleotides and peptides given the knowledge of a person of ordinary skill in the art at the time of filing and the benefit of Applicants' disclosure.

Clearly, Applicants disclose methods of preparing arrays of peptides and oligonucleotides on a solid support in a step-by-step fashion. Examiner Ponnaluri appears to admit this point. It is also clear from the numerous references cited in Applicants' previous response dated 9/23/02 as well as the references cited herewith that those of skill in the art at the time of application were aware of how to make and use arrays of polymers other than nucleotides and peptides on a solid support in a step-by-step fashion. Accordingly, Applicants have fully enabled "polymer arrays" to the extent of its meaning as known by those of skill in the art.

C. A person of ordinary skill in the art could readily monitor arrays of polymers other than peptides and oligonucleotides for their efficiency given the benefit of Applicants' disclosure and the knowledge available to such a person of ordinary skill in the art.

Examiner Ponnaluri is concerned that one would not be able to "control the length" of a polymer other than a peptide or oligonucleotide using the disclosed method, but ***the Examiner has not provided any support*** for this concern. Applicants further disagree because the basic principle of polymer formation remains the same whether the polymer being formed is a oligonucleotide or a polymer other than a peptide or an oligonucleotide, e.g., a polyvinyl

chloride. Applicants disclose these basic principles in the present disclosure and provide examples throughout the specification, e.g., at page 3, line 16 to page 4, line 4. Thus, the cited claims are enabled for the synthesis of arrays of polymers and not just arrays of peptides and oligonucleotides.

Applicants have clearly demonstrated above that the application enables a person of ordinary skill in the art to synthesize an array of diverse polymers, wherein the polymers are polymers other than peptides and oligonucleotides. As such, Applicants respectfully request removal of the present rejection at this time.

VI. Conclusion

Applicants have shown that Lam and/or Holmes neither anticipate nor render the pending claims obvious. Further, Applicants have clearly demonstrated that the specification as filed meets the enablement and written description requirements of § 112, first paragraph.

Having addressed all outstanding issues, Applicants respectfully request allowance of the case at this time. To the extent the Examiner believes that it would facilitate allowance of the case, the Examiner is invited to telephone the undersigned at the number below.

Respectfully submitted,

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SPECIAL REPORT

Combinatorial chemists focus on small molecules, molecular recognition, and automation

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Drug candidates traditionally have been synthesized one at a time, a time-consuming and labor-intensive process. But many researchers in academia, government, biotechnology firms, and drug companies increasingly are turning to combinatorial chemistry - a strategy for creating new drugs that, it is hoped, will speed the drug discovery process significantly.

The idea of combinatorial chemistry is to make a large number of chemical variants all at one time; to test them for bioactivity, binding with a target, or other desired properties; and then to isolate and identify the most promising compounds for further development.

The success of combinatorial chemistry is still uncertain. No drugs discovered combinatorially have been approved for marketing, although several are currently in development. But many researchers believe the technique will prove to be an efficient and cost-effective tool for identifying new medicines.

In combinatorial chemistry experiments, chemical libraries (large collections of compounds of varied structure) are produced by sequentially linking different molecular building blocks, or by adding substituent "decorations" to a core structure such as a polycyclic compound. Libraries may consist of molecules free in solution, linked to solid particles or beads, or even arrayed on surfaces of modified microorganisms.

Combinatorial chemistry initially focused on the synthesis of very large libraries of biological oligomers such as peptides and oligonucleotides. But drug developers generally prefer to focus on small organic molecules with molecular weights of about 500 daltons or less - the class of compounds from which most successful drugs have traditionally emerged. So combinatorial chemistry researchers are concentrating on small organic compounds as well.

Drug discovery is the primary goal of most combinatorial chemistry research, but combinatorial methods also have potential applications for development of advanced materials and catalysts.

One of the challenges of combinatorial chemistry is the difficulty of identifying "hits" (active compounds) present at vanishingly low concentrations in complex combinatorial libraries. To address this problem, ingenious encoding schemes have been developed. Two groups have independently

developed the latest concept in this field - radiofrequency encoding, in which information about library compounds is stored on microchips.

Instrumentation systems to help speed combinatorial chemistry experiments have been developed in-house at a number of biotechnology and pharmaceutical companies. And several combinatorial automation systems are available commercially or undergoing intensive development.

Combinatorial chemistry has come a long way in just a few years, but further advances are needed and new applications are anticipated. Directions in which the field is headed range from combining combinatorial chemistry with computational drug-design strategies to the use of combinatorial molecular recognition for studies of protein function.

Creating libraries

Combinatorial libraries are created in the laboratory by one of two methods - split synthesis or parallel synthesis. In split synthesis, compounds are assembled on the surfaces of microparticles or beads.In each step, beads from previous steps are partitioned into several groups and a new building block is added. The different groups of beads are then recombined and separated once again to form new groups. The next building block is added, and the process continues until the desired combinatorial library has been assembled.

Before split synthesis was developed, explains chemistry professor Kim D. Janda of Scripps Research Institute, La Jolla, Calif., "people created diversity using mixtures of compounds. In a coupling step, you would add, let's say, reagents A, B, and C in one pot, and A, B, and C would all compete to become integrated at the same site. But in doing that you can have problems with kinetics. One reaction may be faster than another and you may not get equal distribution of the three components."

Split synthesis "got away from all that," says Janda. "You could create diversity using separate reactions, so the components would have an equal chance to add in to a site, and then by mixing compounds together again you got the diversity you needed."

Libraries resulting from split synthesis are characterized by the phrase "one bead, one compound." Each bead in the library holds multiple copies of a single library member. Split synthesis greatly simplifies the isolation and identification of active agents because beads (and implicitly individual library members) are large enough to be observed visually and separated mechanically.

Combinatorial libraries can also be made by parallel synthesis, in which different compounds are synthesized in separate vessels (without remixing), often in an automated fashion. Unlike split synthesis, which requires a solid support, parallel synthesis can be done either on a solid support or in solution.

A commonly used format for parallel synthesis is the 96-well microtiter plate. Robotics instrumentation can be used to add different reagents to separate wells of a microtiter plate in a predefined manner to produce combinatorial libraries. Hits from the library can then be identified by well location.

Split synthesis is used to produce small quantities of a relatively large number of compounds, whereas parallel synthesis yields larger quantities of a relatively small number of compounds. And split synthesis requires that assays be performed on pools of compounds, whereas assays on individual compounds can be run on libraries created by parallel synthesis. While slower, testing individual compounds is sometimes advantageous because serious interferences and complications can arise when multiple compounds are tested simultaneously.

A special case of parallel synthesis is spatially addressable synthesis, pioneered by researchers at Affymax Research Institute, Palo Alto, Calif. In this technique, libraries are synthesized in arrays on microchips, and all the compounds on a chip are assayed simultaneously for binding or activity. Hits can then be identified by the piece of real estate they occupy on the chip. Using a chip-making technique called photolithography, Affymax researchers have generated arrays of more than 65,000 compounds on chips about 1 sq cm in area.

Bioactive combinatorial compounds synthesized by split synthesis can also be identified by deconvolution, a technique in which each variable position in a compound library is tested to find the building block that makes the strongest contribution to activity at that site.

Solid-phase and solution-phase combinatorial synthesis each have their advantages and disadvantages. Solid-phase synthesis permits use of excesses of reagents to drive reactions to completion, since excess reagents can be washed away from beads very easily afterward. However, solution-phase synthesis is more versatile because many organic solution-based reactions have not been adapted for solid-phase work.

Janda and coworkers at Scripps recently developed a liquid-phase synthesis procedure that combines some of the advantages of solution-phase and solid-phase synthesis [*Proc. Natl. Acad. Sci. USA,* **92,** 6419 (1995)]. The procedure involves use of polyethylene glycol monomethyl ether in place of solid-phase beads as a foundation for combinatorial assembly. The polymer is soluble in a variety of aqueous and organic solvents, making it possible to use solution-phase combinatorial synthesis. But the polymer can be precipitated out of solution by crystallization at each stage of the combinatorial process to facilitate purifications.

Small-molecule libraries

Combinatorial chemistry began with the synthesis of large libraries of biopolymers such as peptides and oligonucleotides. In some cases, these were created on surfaces of genetically modified microorganisms, such as bacteriophage particles, by inserting combinatorial DNA oligomers into genes that encode cell-surface proteins.

However, peptides and oligonucleotides are problematic for drug development because their oral bioavailability is poor and they are degraded rapidly by enzymes. Hence, the focus of combinatorial research has shifted in recent years to libraries of nonpolymeric small molecules having molecular weights of about 500 daltons or less.

In a pioneering study, chemistry professor Jonathan A. Ellman and coworkers at the University of California, Berkeley, synthesized the first such library by creating variants of benzodiazepines, a class of compounds that has been a fertile source of successful drugs [*J. Am. Chem. Soc.,* **114,** 10997 (1992)]. Since then, researchers have found ways to synthesize combinatorial libraries based on many other classes of small organic compounds.

A recent example is work by Mark A. Gallop, director of combinatorial chemistry, and coworkers at Affymax. They used a cycloaddition reaction to prepare a small-molecule combinatorial library of about 500 mercaptoacyl prolines [*J. Am. Chem. Soc.,* **117,** 7029 (1995)]. By screening this library, they identified an unusually potent inhibitor of angiotensin-converting enzyme (ACE). ACE inhibitors are used as treatments for hypertension and heart disease.

And the group of Stephen W. Kaldor, head of combinatorial chemistry research at Eli Lilly & Co.,

Indianapolis, in collaboration with scientists in Lilly's central nervous system (CNS) group, has used combinatorial chemistry to identify an orally active CNS agent by combinatorial optimization of an existing lead. The low molecular weight nonoligomeric drug candidate entered clinical trials in November. "This is one of the first small-molecule combinatorial compounds to go into humans," says Kaldor.

A major challenge of small-molecule combinatorial chemistry has been to adapt conventional solution-phase organic reactions to reactions on solid-phase particles. Ellman says one of his group's efforts "has been to expand the kind of chemistry that can be performed on solid supports in a simultaneous synthesis format - in particular, carrying out different types of carbon-carbon bond-forming reactions. For example, we've developed general enolate alkylation conditions where side reactions that can be a problem in solution don't occur."

Paralleling the increasing use of small-molecule libraries is a trend toward assaying libraries having smaller numbers of components. "People seem to be much more comfortable working with smaller mixtures - probably a hundred components or less in a mixture, rather than the mixtures of 10^5 and 10^6 compounds per pool that we saw in the early experiments," says Ronald N. Zuckermann, associate director of bioorganic chemistry at Chiron Corp., Emeryville, Calif. "The lower the number of compounds, the more confidence you can have in the biological data" because artifacts arise more readily in the screening of large pools of compounds.

Ellman agrees that "people have gotten away from screening really large mixtures of compounds. They either want to screen them individually or in smaller pools of under 100 compounds. It's easier to extract out binding data in that format."

Oligomers and materials

Carbohydrates have lagged behind other types of compounds in combinatorial library development because of the complexity of oligosaccharide chemistry, but carbohydrate libraries are now beginning to appear. For example, Ole Hindsgaul and coworkers at the department of chemistry of the University of Alberta, Edmonton, in collaboration with researchers at the University of Georgia, Athens, and Ciba Central Research Laboratories, Basel, Switzerland, have developed a "random glycosylation" strategy for making oligosaccharide libraries in solution [*Angew. Chem. Int. Ed. Engl.*, **34**, 2720 (1995)]. They produced a library of all 18 possible fucosylated trisaccharides from disaccharide precursors.

And at a recent meeting, chemistry professor Daniel E. Kahne of Princeton University reported construction of the first solid-phase carbohydrate library, using chemistry for solid-phase synthesis of oligosaccharides developed earlier by his group. This technique has been licensed to Transcell Technologies, Monmouth Junction, N.J. In preliminary work, compounds isolated from one carbohydrate library have been shown to bind a carbohydrate-binding protein with greater affinity than the protein's natural ligand. "Carbohydrates play a central role in some very important biological processes, so having access to libraries of these compounds is critical," says Kahne.

Another type of oligomer being pursued combinatorially is peptoids, peptide analogs that are not recognized by peptide-cleaving enzymes. Chiron researchers recently discovered a candidate urokinase receptor antagonist from a peptoid library, and the compound is currently in preclinical studies as a potential anticancer agent.

"One of the primary advantages of peptoids is their synthetic accessibility," says Zuckermann. "They are efficiently synthesized by the submonomer method, which uses primary amines and bromoacetic acid as

starting materials - both very cheap, and there are literally thousands of amines readily available. The combination of this chemistry with robotic synthesis has led to a truly high throughput synthesis facility."

Chiron's identification of nanomolar peptoids that bind to transmembrane receptors [*J. Med. Chem.*, **37,** 2678 (1994)] "was the first example of the discovery of potent ligands to pharmaceutically relevant receptors from a combinatorial library of nonpeptides or nonnucleic acids - that is, synthetic compounds," Zuckermann adds. "I believe that this work helped inspire others to continue to move away from peptides and further toward small molecules."

Combinatorial chemistry can also be extended entirely beyond the realm of organic chemistry. For example, physicist Xiao-Dong Xiang of Lawrence Berkeley National Laboratory, chemistry professor Peter G. Schultz of UC Berkeley, and coworkers recently devised a combinatorial strategy for finding advanced materials with novel chemical or physical properties - extending "the combinatorial approach from biological and organic molecules to the remainder of the periodic table," as they put it [*Science,* **268,** 1738 (1995)].

Xiang, Schultz, and coworkers used thin-film deposition and physical masking techniques to synthesize libraries of solid-state materials. The properties of the resulting materials were then evaluated to identify promising candidates for further development.

Encoding

In spatially addressable combinatorial synthesis, active compounds can be identified by location. But in other forms of combinatorial chemistry, identifying hits is not so easy because there's often too little of each compound present for characterization with traditional analytical chemistry techniques.

Hence, many researchers now use some form of tagging or encoding to label compounds in large combinatorial libraries. The first such encoding scheme was proposed in 1992 by Scripps President Richard A. Lerner and molecular biologist Sydney Brenner at the institute. They suggested that a combinatorial library could be encoded with oligonucleotides synthesized in parallel with library compounds and linked to each one. Amplification or decoding of the attached oligonucleotide would serve to identify the small molecule bound to each bead.

This idea was independently arrived at and reduced to practice by scientists at Affymax. Later on, researchers at Chiron and at Selectide, Tucson, Ariz., developed similar techniques in which peptides instead of oligonucleotides were used as the sequenceable encoding oligomers.

In 1993, chemistry professor W. Clark Still and coworkers at Columbia University developed a second major type of encoding scheme, in which chromatographically resolvable organic tags were used as encoding elements for bead-based combinatorial libraries. Still devised the technique in response to concerns about the tendency of DNA and peptide tags to break down under the often very rough conditions of organic synthesis.

In Still's technique, inert halogenated aromatic compounds are used to encode the chemical reaction history experienced by each bead. These tags are identified by capillary gas chromatography to reveal the identity of active compounds in the library. Kahne, who used this type of encoding to construct his combinatorial carbohydrate library, says the method "is as good as it gets for identifying hits - a very simple solution to a very important problem."

The most recent development in encoding technology involves the use of radiofrequency tags. Chemistry professor K. C. Nicolaou at Scripps and the University of California, San Diego, together with senior chemist Xiao-Yi Xiao, President and Chief Executive Officer Michael P. Nova, and their coworkers at IRORI Quantum Microchemistry, La Jolla, Calif., developed a technique in which memory devices are associated or coated directly with derivatized polymer during combinatorial synthesis [*Angew. Chem. Int. Ed. Engl.*, **34,** 2289 (1995)]. The chips encode relevant information about the synthetic pathway - including not only reagents used, but also reaction conditions such as temperature and pH. The device can then "report" this information to a receiver via radiofrequency transmission.

"We're putting a manual system to do this type of radiofrequency combinatorial chemistry out on the market in March," says Nova. The system will include radiofrequency memory devices in MicroKans, tiny spherical capsules with porous walls that also enclose polymer beads for combinatorial synthesis.

A related technique was developed independently by synthetic chemist Edmund J. Moran and coworkers at Ontogen Corp., Carlsbad, Calif., and the University of California, Los Angeles [*J. Am. Chem. Soc.*, **117,** 10787 (1995)]. This approach differs from the Scripps technique in that reaction data from each stage of combinatorial synthesis are stored in a computer database, rather than being retained in the chip itself. An identification number stored in the memory of each chip is a pointer to reaction information in the database. Moran and coworkers have applied the strategy successfully to the discovery of novel inhibitors of a protein tyrosine phosphatase.

Molecular recognition

A combinatorial chemistry application that has become increasingly active in the past year or so, and that promises to grow even more rapidly in the future, is combinatorial molecular recognition - the use of combinatorial techniques to study binding between biological or synthetic receptors and their ligands. Researchers in the combinatorial molecular recognition community "want to be able to make small molecules that do the kinds of things that antibodies do - tightly and selectively bind important molecules or transition states," explains Columbia's Still.

"We made libraries of substrates just to measure the binding properties of compounds synthesized as enantioselective receptors," says Still, "and the receptors did indeed have significant sequence-selective binding properties that had never been observed before." The results of such experiments suggest, says Still, "that virtually anything people can do with antibodies ought to be doable with small molecules, and that it may not be that hard to identify small molecules that are as selective as antibodies for binding substrates."

Chemistry professor Stuart L. Schreiber and coworkers at Harvard University are also using combinatorial molecular recognition - in this case in conjunction with nuclear magnetic resonance spectroscopy (NMR) - to study protein receptors. They have focused initially on the SH3 domain, a frequently occurring structural feature in proteins (such as tyrosine kinases) involved in signal transduction.

The researchers identified peptide ligands that bound SH3 in two binding pockets that make up part of the SH3 binding site. The SH3 binding site also includes a third binding pocket that is highly variable in structure and is therefore referred to as a "specificity pocket." A combinatorial strategy led to the discovery of two classes of peptide ligands that bind to the three pockets in opposite orientations, as determined by NMR analysis of the SH3-ligand complexes. Last month, Schreiber and coworkers reported also having identified nonpeptide elements that bind to the specificity pocket [*J. Am. Chem. Soc.*, **118,** 287 (1996)].

Chemistry professor Fredric M. Menger and coworkers at Emory University, Atlanta, are also using a form of combinatorial molecular recognition - in this case to identify industrial catalysts [*J. Org. Chem.*, **60,** 6666 (1995)].

"Libraries have in the past been screened for noncovalent binding," says Menger. "But this is the first, or one of the first, cases where purely organic libraries have been investigated for catalytic activity. We make hundreds or thousands of compounds very quickly and then test their catalytic power. The potential catalysts are polymers that have multiple functional groups in different proportions and different sequences, plus a metal ion."

In screening for catalytic activity, "we selected the hydrolysis of a phosphate ester," says Menger, "but one could choose any reaction of interest. Once a polymer with activity is found, we begin tinkering with the proportions to fine-tune it until it gets faster and faster."

Using this approach, Menger and coworkers have identified polymers that accelerate phosphate hydrolysis by a factor of 10^4 or more. According to Menger, "The potential exists for even greater acceleration ... since only a small portion of the vast number of possible combinations has as yet been tested."

In future work, the researchers hope to make chiral polymers that can reduce functional groups enantioselectively. "I would not be surprised if in 10 years most new catalysts are developed combinatorially," says Menger. "Industry is moving more and more toward aqueous systems to avoid organic solvents. If one could devise catalysts for organic reactions in water, that would be a useful practical development."

Automation

Planning and performing combinatorial experiments in the laboratory is a complex and potentially tedious process. Hence, "A future trend is going to be greater availability of automation devices," says Chiron's Zuckermann. "A lot of solutions are being developed for automating combinatorial split synthesis or multiple parallel synthesis."

For example, Chiron has developed proprietary robotic combinatorial synthesizers. "We now have third-generation units working in our labs that feature all-glass reaction vessels, heating to 120°C, and flexible software, [allowing] automation of most organic reactions," says Zuckermann.

Ontogen has developed OntoBLOCK, an in-house combinatorial chemistry automation system that can produce 1,000 to 2,000 small organic molecules per day by parallel array synthesis. The system includes reaction blocks containing 96 reaction vessels, from which compounds can be transferred directly to standard 96-well microtiter plates for high-throughput screening.

Bohdan Automation Inc., Mundelein, Ill., markets a combinatorial chemistry reaction block that accommodates a wide variety of organic solvents and handles both solid-phase and solution-phase chemistry. Advanced ChemTech, Louisville, markets instrumentation for combinatorial peptide and organic synthesis. And Tecan U.S. Inc., Research Triangle Park, N.C., offers an organic chemical synthesizer called CombiTec that includes a robotic sample processor and reaction blocks of eight to 56 chambers.

Robotics maker Zymark Corp., Hopkinton, Mass., has put together several different automation systems that enable their clients to do solution-phase combinatorial synthesis and solid-phase peptide and

peptoid synthesis. The reactions can generally be performed under inert gas at a variety of temperatures.

According to Brian Lightbody, general manager of drug discovery business development at Zymark: "The process of generating combinatorial compounds involves several steps in addition to the actual reaction - [including] initial formulation of the reactants, labeling, pooling and splitting, cleavage, liquid-liquid extraction, solid-phase extraction, and evaporation. These steps require extensive manual labor. ... An automated robotic approach can often be implemented to fulfill these requirements, dramatically reducing the manual labor and eliminating the sources of human error."

A combinatorial chemistry system still in the prototype stage is the Nautilus, a synthetic chemistry workstation being developed by Argonaut Technologies Inc., San Carlos, Calif. The instrument handles a wide range of reagents, with capabilities for temperature control and use of inert atmospheres.

"The Nautilus allows you to do pretty much what you're able to do on the bench except in an automated fashion," says Argonaut President and CEO Joel F. Martin. "The system is completely enclosed and encapsulated, with a pressurized fluid delivery system and no exposure to the atmosphere whatsoever. It's a closed system, and all wetted surfaces within the instrument are glass or [polytetrafluoroethylene, such as DuPont's] Teflon."

Procedures that have been demonstrated on the Nautilus include a Suzuki coupling (a carbon-carbon bond-forming reaction at elevated temperature using an air-sensitive palladium catalyst), a butyllithium reaction, enolate reactions of the type developed by Ellman and coworkers, and synthesis of a solid-phase druglike molecule. "We chose tough organic reactions that no one would ever have conceived of doing in an automated synthesizer in the past," says Martin. The Nautilus is scheduled to be released commercially in August.

CombiChem Inc., San Diego, is developing commercial instrumentation for combinatorial chemistry that is likely to be competitive with the Nautilus. "Every company now is looking at ways of automating synthesis, purification, and analysis," says CombiChem Chief Operating Officer Peter L. Myers. "There's a major revolution going on. It's probably not obvious to a lot of people, and the academics may think we're overemphasizing it. But I know for a fact that every company now is looking to automate combinatorial chemistry because chemistry's become the rate-determining step."

As to whether conventional robotics instrumentation can be used effectively for combinatorial chemistry synthesis, "This immediately gets one into a debate," replies Myers. "When you're doing chemical reactions to make small molecules, many of the reactions are sensitive to conditions such as the presence of water vapor or oxygen, so inert atmospheres such as argon and nitrogen are often needed. The only successful way of blanketing a reaction is to have a closed system - one that is sealed. And if you seal it, then of course you can't use a robot very easily."

Myers adds, "This is why we, and also Argonaut, have gone to nonrobot systems - closed systems that work on valves and plumbing. ... That essentially means individual reaction vessels presealed with a solid support or chemicals inside, delivery by valving, and some way to agitate or stir the contents. Then you let the reaction proceed and wash the resin at the end, if it's a solid-phase reaction." However, he concedes that many researchers are currently using robotic systems instead of closed systems for combinatorial synthesis, "so the jury is out on which is the most acceptable."

CombiChem's instrument will be capable of automating both solid-phase and solution chemistry. "If you really want to exploit as much diversity as you can ... you have to be able to do something in addition to just solid-phase chemistry," says Myers. "The reason is pretty obvious. There are about 150 reactions

now that work on solid phase. Some of those reactions work extremely well, some are still very poor yielding. But the organic chemists have an armory of thousands of chemical reactions that have been developed over the years, and of course primarily most of those are done in solution."

3-Dimensional Pharmaceuticals Inc., Exton, Pa., is also developing combinatorial chemistry instrumentation. The system is based on a technique called DirectedDiversity, an iterative optimization process that explores combinatorial space through successive rounds of selection, combinatorial synthesis, and testing. In each step, a chemical library is generated by robotic instruments, structure-activity information is obtained on library members, and data are analyzed to determine how closely the synthesized compounds match a set of desired properties. In each succeeding iteration, the structure-activity models are refined and new compounds are created until desired drug leads have been identified.

Future needs and prospects

Combinatorial chemistry has come a long way in the past few years, but many challenges still lie ahead. For example, Ellman foresees further development of solid-support chemistry, including new linkage strategies and novel methods for synthesizing support-bound libraries and cleaving compounds from supports. "And people will continue to focus on different types of templates - novel templates for the versatile display of functionality," he says.

Ellman also believes "there are some interesting opportunities in the area of combining combinatorial strategies with computational strategies and structure-based design. The idea is to use information about three-dimensional structures of receptors and enzymes in combination with libraries to rapidly identify high-affinity ligands. It is going to be interesting to see how best to combine these two approaches." The recent SH3 study by Schreiber's group exemplifies this strategy of using a knowledge of protein and protein-ligand structure to help design optimal libraries.

Eric M. Gordon, vice president of research and director of chemistry at Affymax, points out that "some people enter into the molecular diversity sphere with the idea that it's a random process and that what you want to do is make as many molecules as you can that are as different from each other as they can be, but that no particular thought has to go into the design of these libraries. That's an extreme position. I believe that combinatorial chemistry doesn't stand alone. It should be integrated in with the arsenal of tools used for drug discovery, as opposed to being viewed as a competitive technology, say with structure-based design. I think the fate of it and the greatest power of it is going to be when it's used in concert with structure-based approaches and computational approaches."

Still believes that future prospects for combinatorial chemistry are good. "A lot of drugs will be discovered with it," he says. "And it's hard to imagine that there will not be people who find some enormously interesting catalytic compounds and stoichiometric reagents using these methods." However, he says, "The real key to making it work is twofold. First, you really need to have a good idea - a good basic structure that has a real chance of doing something really interesting, and you want to manufacture that idea in as many variants as you can afford to screen."

A second and even greater challenge, says Still, is devising novel and effective assays. "You need assays for the property you want that can be run in parallel, so you can select the beads or the library members that you want just by simple inspection. You simply look at them and pull out the ones that have the right property."

Gordon agrees that greater assay development is needed. "The amount of molecular diversity and the number of molecules that are going to become available are going to dwarf the present screening

capacities," he says. "What's required is more individualized assays and assay miniaturization."

Miniaturization not only saves on the cost of proteins and other rare materials used in assays but also provides greater compatibility with the very small amounts of combinatorial compounds that are typically synthesized on beads. "Instead of 96-well microtiter dishes, which are the current standard in the pharmaceutical industry, you're going to see 1,000-well trays," Gordon predicts.

Ontogen's Moran believes that an increased focus on analytical chemistry is needed in the combinatorial field. "One needs to produce a reasonable amount of material in order to characterize any one compound that might be of interest in a library, either by mass spectroscopy or more preferably by proton NMR," says Moran. "The reason for this is that organic synthesis is not straightforward."

Different groups added to a core structure will affect the reactivity of library members to a differential extent, leading to possible failures of key synthetic steps. "So one needs to get a handle on how much of each component is being produced and whether you're actually making all the components in your library," he says. "Unless we have good analytical control over our experiments, it's going to be a challenge knowing what one's made." However, another researcher comments that, although it's important to be able to analyze hits, it's not practical or even desirable to analyze all library compounds.

Janda believes another key goal for the future is "to create a global library or universal library, where if you screen the library you'd find a hit for any type of target. It might not be a very potent hit, but you'd find a lead. People are trying to create a small library that would be very diverse that would give you leads to almost anything in the drug area. Some people call this combinatorial chemistry's Holy Grail."

However, Still says the universal library may prove to be as permanently elusive as the Holy Grail. In combinatorial chemistry, he says, "medicinal chemists ... design the first library of 1,000 to 100,000 compounds that has a good chance of acting on the target they are going after. Then they screen and make a new sublibrary based on the structure-activity relationships they find. I don't think any medicinal chemist would believe any single library of 10,000 compounds, no matter how carefully chosen, will contain leads for every medical target."

Schreiber suggests that combinatorial molecular recognition could become a fundamental tool for understanding protein function. One of the ultimate goals of the Human Genome Project is to discover the functions of human proteins, he says, and up to now this has been done with molecular biology techniques.

"Virtually all studies of the functions of proteins today involve making mutations in the genes that encode proteins and studying the effects," he explains. "This genetic approach to studying protein function is very powerful, but it is very slow and very inefficient. It's going to take centuries to study the function of all the proteins encoded by the human genome this way, and that's simply unacceptable."

In principle, this problem could be solved, he says, by using a "chemical genetics approach - where instead of making mutations in the gene encoding the protein you attack the protein itself by using organic ligands that bind to it." And such ligands can best be identified with combinatorial methods.

Hence, says Schreiber, "Chemical genetics could be the way in the future to solve the problem of protein function. There's a big advantage if you do it that way - because the very act of understanding protein function gives you a molecule that actually alters function. In terms of medical applications of the knowledge we seek, that's what one is ultimately trying to do."

Combinatorial chemistry, coupled to structural biology and cell biology, "is the most likely avenue to solve the protein binding problem," he says. "If we can combine those techniques, the consequences will be very exciting. It will lead to an era where biology is intimately coupled to chemistry, and where one might even say that chemistry, rather than genetics, will drive biology."

The ultimate usefulness of combinatorial chemistry for drug discovery and other applications remains to be proved. But Lilly's Kaldor - whose group developed by combinatorial means the CNS agent that has advanced to the clinic - is one researcher who is cautiously optimistic. "These techniques are more broadly applicable than crystal-structure-guided design methods because you don't have to have any knowledge of your receptor in order to apply them. ... You can develop a pharmacophore hypothesis much more quickly than you might have otherwise been able to do so. To date, we have used combinatorial chemistry for lead generation or lead optimization in over 50% of current Lilly projects and anticipate this percentage will increase with time."

Lilly's development of the CNS compound took less than two years from target identification to the beginning of clinical trials. This is "very fast," says Kaldor, "and we, of course, are being challenged by our management to repeat this success in every project we work on. ... It's a stunning example of what can be done if ... you apply combinatorial chemistry."

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Split-Pool Method for Synthesis of Solid-State Material Combinatorial Libraries

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The synthesis and analysis of inorganic material combinatorial libraries by the split-pool bead method were demonstrated at the proof-of-concept level. Millimeter-size spherical beads of porous γ-alumina, a commonly used support material for heterogeneous catalysts, were modified with $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ cations in order to promote irreversible adsorption of the anionic fluorescent dyes Cascade Blue, Lucifer Yellow, and Sulforhodamine 101. The compositions of individual beads were easily determined through three split-pool cycles using a conventional fluorescence plate reader. Small split-pool material libraries were made by adsorbing noble metal salts (H_2PtCl_6, H_2IrCl_6, and $RhCl_3$) into the beads. Analysis of these beads by micro-X-ray fluorescence showed that quantitative adsorption of metal salts without cross-contamination of beads could be achieved at levels (0.3 wt % metal loading) relevant to heterogeneous catalysis. The method offers the potential for synthesis of rather large libraries of inorganic materials through relatively simple benchtop split-pool chemistry.

Introduction

Combinatorial synthesis and screening are now well-established tools for the discovery of solid-state inorganic materials. In a sense, this is the oldest application of the combinatorial method, dating back over 90 years to the work of Mittasch on catalysts for ammonia synthesis.[1] His experiments systematically explored combinations of catalysts, supports, and promoters using macroscale reactors. The method was later refined in the continuous phase spread approach of Hanak[2] and in the discrete combinatorial libraries developed by several groups.[3] All of these groups used planar arrays of microscale samples, enabling more rapid searches through larger numbers of compositions. These high-throughput methods have been used in the past several years to search for better superconductors,[2,3a,b] heterogeneous catalytic materials,[3c-g,4] electrocatalysts,[5] phosphors,[6] dielectrics,[7] and sensor materials.[8] Combinatorial methods are likely to continue to be used in areas of materials research in which improvements and new discoveries are most effectively made by combining heuristic chemical ideas with an Edisonian mapping of the relevant parameter space.

Until now, almost all combinatorial work on inorganic materials has employed spatially addressable libraries. These are continuous or discrete arrays in which composition and/or synthetic parameters are systematically varied. An important attribute of these libraries is that every member is uniquely identified by its position in the array. Correlating the performance of a given material with its composition or processing history is therefore quite straightforward. Spatially addressable material libraries have three important drawbacks, however. One is that the size of a planar array of materials is usually limited in the practical sense to a few hundred members. A second problem is that the cost of the synthetic equipment can be high, particularly if lithographic or high-vacuum vapor deposition techniques are needed to fabricate the array. Third, it can be difficult, particularly with dense arrays, to incorporate materials in the physical form in which they are used in the "real world". For example, heterogeneous catalysts are often made as pelletized, highly porous composites that do not lend themselves particularly well to synthesis and screening in planar arrays.

Bead libraries have been widely used in bioorganic combinatorial chemistry.[9] Their synthesis is straightforward, and very large libraries can be made quickly and inexpensively. In the split-pool method,[10] a collection of small polymer beads is split into vials and a different component (e.g., an amino acid) is added to each vial. After all the reactions are complete and excess reagents have been removed, the beads are mixed together and then split again into separate vials. The whole process is repeated several times to construct the library. A chemical or physical tagging process often accompanies the split-pool steps to aid in later identification of individual beads or of collections of beads that are processed together in each step as a "tea bag." An important property of the split-pool method is that only one compound (e.g., a single polypeptide sequence) is synthesized on a particular bead or in a particular tea bag.

In bioorganic libraries, the identity and properties of these combinatorially synthesized molecules depend on the order in which components are added to the beads. The number

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of unique compositions in the library is therefore n^m, where n is the number of components (the same as the number of vials) and m is the number of split-pool operations. For material libraries, the order of addition of reagents may or may not be important because postsynthesis thermal treatments can effectively mix all the components. Planar material arrays are therefore normally prepared and screened without regard for the order of addition of reagents. In the simplest case, in which the order of addition does not matter, the number of different bead compositions N is given by

$$N(n,m) = \frac{(n+m-2)!}{(n-1)!(m-1)!} \quad (1)$$

It is apparent from eq 1 that the number of different members of a split-pool material library can become larger, in a relatively small number of simple steps, than that accessible in a very sophisticated planar array. For example, with 10 different components and 8 split-pool steps, one obtains $N(10,8) = 11\,400$ discrete compositions. A caveat in preparing such a library is that the inorganic reagents and tags (if a tagging scheme is used) must be adsorbed uniformly and irreversibly onto the beads in each synthetic step. Further, one needs a technique for rapidly identifying and screening the beads for the particular property of interest.

In this paper, we describe the split-pool synthesis of an inorganic material library. For this proof-of-concept example, we chose components (noble metals) and bead supports (porous γ-alumina) that represent realistic choices for a library of heterogeneous catalysts. In addition, we have examined the adsorption of fluorescent dye tags, which might be used for postsynthesis bead identification, on the same supports by the split-pool approach.

Experimental Section

Materials. $H_2IrCl_6 \cdot xH_2O$, $RhCl_3$, $HAuCl_4$, H_2PtCl_6, and $RuCl_3$ were purchased from Alfa Aesar and used as received. Cascade Blue, Lucifer Yellow, and Sulforhodamine 101 fluorescent dyes were purchased from Molecular Probes, Inc. and used as received. The support beads were composed of γ-alumina and had a surface area of 200 m^2/g and an average diameter of 1 mm. The average mass of the support beads was 2.3 mg. All other chemicals were analytical grade and were used as received from commercial sources.

Solutions of the aluminum Keggin ion, $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ (Al_{13}^{7+}), were prepared by reaction of the sulfate salt with aqueous $BaCl_2$.[11] An amount of 50 mL of an aqueous 0.25 M NaOH solution (12.5 mmol) was added dropwise to a solution of 1.2 g of $AlCl_3 \cdot 6H_2O$ (5 mmol) in 50 mL of water. The resulting solution was heated to 85 °C in an oil bath with constant stirring. After 20 min, 80 mL of aqueous 0.12 M Na_2SO_4 (10 mmol) was added. The solution was kept at room temperature for 1 day to yield crystals of the sulfate salt $NaAl_{13}O_4(OH)_{24}(SO_4)_4 \cdot xH_2O$. The crystals were separated by suction filtration, washed with deionized water, and dried. The dry crystals (0.25 g, 0.18 mmol) were resuspended in 100 mL of deionized water. $BaCl_2 \cdot 2H_2O$ (0.28 g, 1.1 mmol) was added to the suspension, which was stirred for 4 h. The $BaSO_4$ produced in the reaction was removed by filtration and centrifugation. The resulting aqueous solution contained Al_{13}^{7+} and Ba^{2+} at approximately 1.8 and 3.8 mM concentrations, respectively.

Modification of γ-Alumina Beads with Al_{13}^{7+}. The surface of the γ-alumina beads was made cationic by adsorption of Al_{13}^{7+} ions. The γ-alumina beads (0.150 g) were reacted overnight with 15 mL of Al_{13}^{7+} solution (ca. 1.8×10^{-3} M). The solution was then removed, and the beads were thoroughly rinsed with water and then dried at room temperature in air.

Synthesis of Dye Libraries. In a typical procedure, 15 Al_{13}^{7+} modified γ-alumina beads were equilibrated in a small vial with 2 mL of Cascade Blue (4.2×10^{-8} M), Lucifer Yellow (5.5×10^{-8} M), or Sulforhodamine 101 (8.3×10^{-9} M) solutions in water. The adsorption step took 40 min, and after that the beads were rinsed with water, they were dried at room temperature for 1 h and then dried in vacuo overnight. The beads were then combined (pooled) and distributed equally into three vials (split). The adsorption steps were repeated until the desired loading was achieved. In directed sorting experiments, the fluorescence intensity of the individual beads was measured between split-pool steps (see below).

Synthesis of Noble Metal Libraries. In split-pool syntheses, 350 mg of unmodified γ-alumina beads were reacted overnight in small vials with 1 mL of H_2PtCl_6, H_2IrCl_6, or $RhCl_3$ aqueous solution in sufficient concentration to make the final loading 0.1 wt % in metal. The extra solution was then withdrawn and was colorless, meaning that most of the metal ions had been adsorbed onto the beads. The beads were dried at 120 °C for 2 h and then at 300 °C for 3 h. The latter thermal treatment was needed with these particular metal salts in order to minimize desorption in subsequent steps. The beads were allowed to cool to room temperature and were then collected together, mixed, and divided into three parts. The adsorption and thermal treatment steps were repeated until the desired loading was achieved.

In control experiments used to calibrate analytical procedures for metal-loaded beads, individual beads were impregnated with measured amounts of aqueous metal salt solutions delivered by a robotic plotter. Five metal halide solutions (H_2IrCl_6, $RhCl_3$, $HAuCl_4$, H_2PtCl_6, and $RuCl_3$) were prepared so that 9 μL of solution (the total amount delivered to each bead) contained 0.0113 mg of metal. Each metal salt was dissolved in approximately 20 mL of water and 0.531 mL of concentrated hydrochloric acid. The solutions were heated to boiling for 15 min, cooled to room temperature, and then diluted to 25 mL. The plotter (Cartesian Technologies, PixSys 3200) was programmed to deliver the metal salt solutions into two 384-V-bottom-well plate (Nalge Nunc International) arrays. One alumina sphere was placed manually into each well. The plotter delivered 11 μL of water to each well. A total of 9 μL of metal salt solutions was added to each well to make 715 distinct compositions ($=N(5,10)$) at a resolution of 10 compositions along each binary edge in the pentanary composition map.[5b,8b] The remaining 53 wells were programmed as duplicates of a single pentanary composition, $Pt_5Ru_3Au_1Ir_1Rh_1$. The two 384-well plate arrays were dried overnight on an orbital shaker.

Fluorescence of Dye-Tagged Beads. Fluorescence measurements were made with an HTS 7000 Plus bioassay reader (Perkin-Elmer). The beads were first loaded into a 96-well V-bottom plate. Measurements were then carried out using the appropriate excitation/emission filter set for each of the three dyes. In this mode of operation, the plate reader took approximately 25 s to record the emission intensities of a single dye on 96 beads.

Elemental Analysis. Metal loadings on representative beads were determined using micro-X-ray fluorescence spectroscopy. The samples were analyzed using a ThermoNORAN Omicron system, which consisted of a microfocus X-ray source operating at 100 W with a molybdenum target X-ray tube, a selection of eight filters, and a 175 eV liquid-nitrogen-cooled Si(Li) detector, coupled to an ADC. A 100 μm collimator was used. Samples were presented to the X-ray beam using a custom tray mounted on an automated precision *XYZ* stage. A PC controlled the setup, automation, and data analysis through a Windows-based software. No special sample preparation was necessary. However, to minimize geometrical effects, care was taken to mount the beads on a level surface and to measure each bead at its center. The source was operated at 40 kV, 2.0 mA, in a vacuum with an acquisition time of 600 s. Net peak intensities were extracted using Gaussian peak-fitting together with digital background correction, which deconvolutes overlapping peaks such as those of Ir, Pt, and Au. The absolute concentrations were determined using a linear least-squares calibration.

Results and Discussion

Dye Libraries on γ-Alumina Support Beads. The split-pool principle can be demonstrated for catalyst support beads using fluorescent dyes as the adsorbate. In principle, these and other anionic dyes could be used in tagging schemes to represent the metal ions adsorbed onto the beads in the same split-pool cycle. The prerequisite for use of these fluorescence dyes is that they should have distinct excitation/emission signatures and that their adsorption must be quantitative and irreversible.

Three commercial anionic dyes (Figure 1) were used in these experiments. These dyes have previously been used in combination for three-color mapping of neuronal processes.[12] Adsorption of these dyes onto unmodified beads gave easily detectable fluorescence at loadings in the range of 1.8×10^{-11} mol per bead (or approximately 3.8×10^{-15} mol per cm^2 of support area). However, an unacceptable level of desorption was observed in subsequent split-pool steps with unmodified beads. To increase the affinity of the dyes for the beads, the beads were first modified with $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ (Al_{13}^{7+}) cations. The coverage of Al_{13}^{7+} ions was 4.3×10^{-7} mol per bead (approximately 9.0×10^{-11} mol per cm^2 of support area, or roughly $^1/_3$ monolayer coverage, assuming that the diameter of the Al_{13}^{7+} ion is 7 Å). Beads with different dye loadings were then prepared by adsorption of the dyes from aqueous solutions. The optimum loading (ca. 7×10^{-12} mol per bead), which is high enough for detection while low enough that concentration quenching and energy-transfer quenching do not occur,

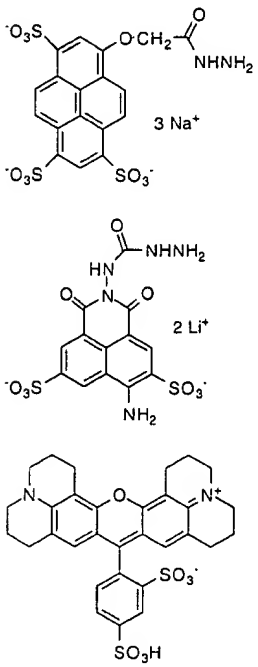

Figure 1. Molecular structure, absorbance and emission maxima, and filter sets used to measure fluorescence intensities of anionic dyes adsorbed onto Al_{13}^{7+}-modified γ-alumina beads.

was then determined using a Perkin-Elmer HTS 7000 bioassay reader. This loading corresponds to saturation of approximately 0.002% of the available Al_{13}^{7+} sites with dye molecules. Using V-bottom 96-well plates, the beads were reliably located in the center of each well, which is critical for reproducible detection of fluorescence intensities.

Table 1 shows the raw fluorescence intensity signals obtained from rows of beads loaded with the three dyes and examined with the filter set appropriate for Sulforhodamine 101. The mean value is 334 counts, and the standard deviation is 23. Only one of the intensity measurements is outside the range of 2 standard deviations, which is 14% of the mean. Similar results were obtained for the second and third rows using appropriate filter sets. For beads loaded with dyes that did not match the filter set used, the detected fluorescence was not significantly different from that measured with control beads containing no dye. This implies that the resolution offered by these dyes as tags is sufficient to discriminate approximately $334/(2 \times 23) = 7$ concentration levels of each "active" component delivered in the same split-pool step. Since a tag is not needed in the last step, libraries could be made in up to eight split-pool steps with fluorescent identification of most of the beads.

The error in the fluorescence measurements can be attributed primarily to the variability in bead size, as shown in Figure 2. In this experiment, a selection of large, small, and average size beads was chosen; that is, the distribution of weights shown in the figure is not representative of the entire sample, which had a higher proportion of average-size beads. The plot shows the expected trend of increasing fluorescence intensity with bead mass. The optical micrograph of the beads confirms that there is some variability in bead diameter. This problem could be partially addressed by sorting the beads manually.

Table 1. Raw Fluorescence Intensities of Rows of Beads Loaded with a Single Dye[a]

	bead no.											
dye	1	2	3	4	5	6	7	8	9	10	11	12
SR 101	307	329	328	334	357	320	344	321	341	310	330	391
Cascade Blue	0	0	7	1	0	1	0	2	3	2	3	4
Lucifer Yellow	2	2	2	5	0	0	2	2	2	1	3	1
none	0	0	3	2	2	0	1	1	0	4	2	1

[a] The excitation/emission filter set for detection of Sulforhodamine 101 was used for all beads. Comparison with a row of Al_{13}^{7+}-modified beads containing no dye shows that the background signal from Cascade Blue and Lucifer Yellow loaded beads is minimal.

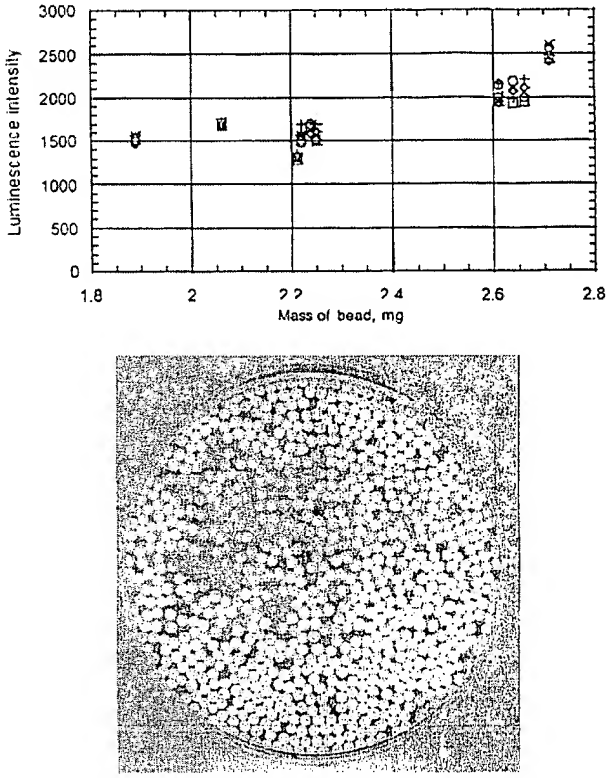

Figure 2. (Top) Fluorescence intensity of Al_{13}^{7+}-modified γ-alumina beads loaded with Lucifer Yellow as a function of bead weight (in mg). (Bottom) Optical micrograph of γ-alumina beads.

A proof-of-concept split-pool library was prepared by adsorbing the three dyes into three separate vials, rinsing, drying, and pooling the beads, and then repeating until three adsorption cycles had been completed. Figure 3 shows normalized fluorescence intensities from this small dye library using the Sulforhodamine 101 (red) filter set. The rows in this small library are color-coded according to the dye adsorbed in the last split-pool cycle; that is, each color corresponds to a set of 15 beads that were pooled in the last adsorption step. For beads onto which the last dye adsorbed was Cascade Blue or Lucifer Yellow, the number of possible Sulforhodamine 101 adsorption steps was 0, 1, or 2. Likewise, for beads onto which Sulforhodamine 101 was adsorbed in the final step, the possibilities are 1, 2, and 3. The normalized intensity data in Figure 3 are consistent with these expectations, showing "0" values only in the blue and yellow rows and "3" values only in the red row.

By repetition of this analysis with the blue and yellow filter sets, each of the beads can be identified with the number of red, blue, and yellow adsorption steps. The total should equal the total number of split-pool cycles for each bead. Normalized intensity data for this type for a 45-bead, three-step split-pool library are shown as three-digit numbers in Table 2. Note that for each of the 45 beads, the total is 3, indicating that each one is unambiguously identified. Because this is a random library, there is a random distribution of compositions, the most common (10 beads) being the ternary (111) and the least common being the single dye compositions (300), (030), and (003), with 2, 3, and 1 beads, respectively.

One problem with the split-pool technique is that a large number of beads must be used to ensure that the whole range of compositions is represented by at least one bead. A random library generates many redundant beads, as illustrated by the binary and ternary combinations shown in Table 2. A more economical library, with fewer redundancies, can be designed by directed sorting. This method involves identification of each bead by fluorescence intensity measurements after the second and subsequent split-pool steps. The directed sorting scheme is illustrated in Figure 4 for a 36-bead library prepared in three steps, and the resulting fluorescence data are shown in Table 3. For 35 out of 36 beads, the fluorescence data match the library design. These data show that the desired number of beads of each composition can be prepared with a precisely controlled degree of redundancy.

Noble Metal Libraries. Dye libraries are convenient for illustrating the idea of sequential synthesis on inorganic support beads, and they can in principle be used as fluorescent markers for inorganic substances adsorbed in the same split-pool cycle. However, dye libraries themselves are not particularly interesting as materials. On the other hand, transition metals and metal oxides supported on alumina beads resemble the materials used in heterogeneous catalysis and other applications. Typically, these materials are prepared from metal halide salts by adsorption or impregnation. We therefore prepared several bead libraries by these techniques and used single-bead X-ray fluorescence (XRF) to analyze the results.

Table 4 shows single-bead XRF results for ternary compositions selected from a 715-member pentanary bead library prepared by simple impregnation. The nominal compositions in this (spatially addressable) array are known a priori, and the purpose of this experiment was to gauge the accuracy of the single-bead analysis. The agreement in the Pt, Rh, and Ir columns is generally quite good, and certainly good enough to be able to identify individual bead compositions. The scatter in the Au analysis was traced to nonuniform impregnation.

Supported Pt, Rh, and Ir are widely used in heterogeneous catalysis and therefore represent interesting candidates for

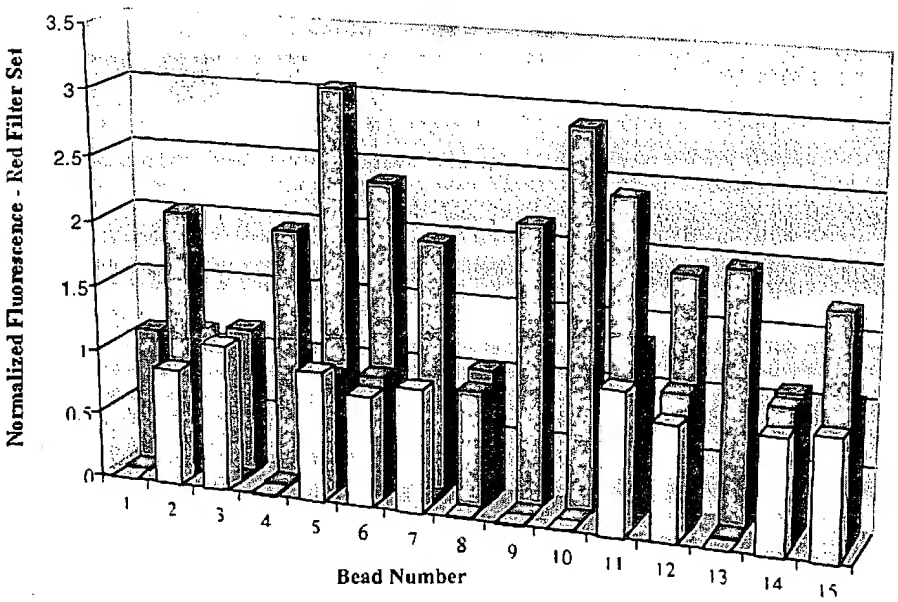

Figure 3. Normalized fluorescence intensities for a dye-loaded bead library prepared in three split-pool cycles and analyzed using the Sulforhodamine 101 (red) filter set. The color code indicates the dye adsorbed in the final split-pool cycle. The data were obtained from 45 beads arranged in three lanes of 15 beads each.

Table 2. Fluorescence Data for a 45-Bead Library after Three Split-Pool Cycles[a]

final dye adsorbed	bead composition (red, blue, yellow)														
SR 101 (red)	111	120	111	201	300	201	210	102	210	300	111	210	210	111	111
Cascade Blue	030	210	021	012	120	120	030	111	012	030	210	111	012	120	210
Lucifer Yellow	012	102	111	012	102	102	102	021	012	003	102	102	012	111	111

[a] Sulforhodamine 101, Cascade Blue, and Lucifer Yellow were adsorbed in the last cycle onto beads in the first, second, and third rows, respectively. Three-digit numbers indicate the normalized intensities using the red, blue, and yellow filter sets. For example, "210" indicates two adsorption steps with Sulforhodamine 101, one with Cascade Blue, and zero with Lucifer Yellow.

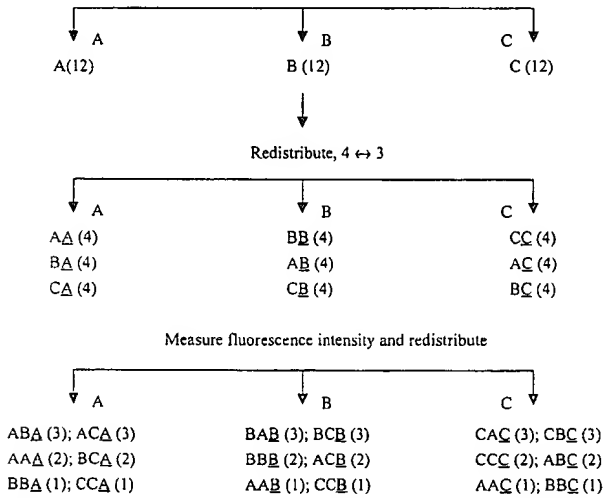

Figure 4. Directed sorting split-pool scheme. The numbers in parentheses indicate the number of beads of each type.

the synthesis of split-pool bead libraries. As with the dye-loaded beads, we prepared a small random library in three split-pool steps. The concentrations of the metal solutions (H_2PtCl_6, $H_2IrCl_6 \cdot xH_2O$, and $RhCl_3$) were sufficient to add a loading of 0.1% metal to the beads in each adsorption step, meaning that the theoretical total loading was 0.3%. This library should give 10 different compositions: Pt_3, Rh_3, Ir_3, Pt_2Rh_1, Pt_2Ir_1, Pt_1Ir_2, Pt_1Rh_2, Ir_1Rh_2, Ir_2Rh_1, $Ir_1Pt_1Rh_1$ (subscripts indicate the number of adsorption steps in each metal ion solution). XRF analysis of representative beads from this library are shown in Table 5. With this simple library, and with more complex compositions (as shown in Table 4), the XRF technique is sufficiently accurate to determine the total number of adsorption steps of each metal on each bead. It is worth noting that, within the detection limits of XRF, none of these beads contain all three elements. From the absence of the third element, we can conclude that there is minimal desorption and mixing of metal ions after three split-pool steps, provided that the beads are heated to decompose the adsorbed metal chlorides between steps.

Table 3. Bead Compositions for a Directed Sorting Library Prepared in Three Split-Pool Cycles[a]

compositions	experimental design	experimental results
300	2	2
030	2	2
003	2	2
210	4	4
201	4	3
120	4	4
021	4	4
102	4	4
012	4	4
111	6	6

[a] The target distribution of beads matches the normalized fluorescence data with one exception.

Table 4. X-ray Fluorescence Analysis of Individual γ-Alumina Beads Prepared by Impregnation with Metal Salt Solutions in V-Bottom Well Plates

expected composition				experimental composition			
Pt	Au	Rh	Ir	Pt	Au	Rh	Ir
0	4	2	3	0.1	2.4	1.3	2.3
3	0	2	4	3.1	0.9	1.6	3.8
5	2	2	0	4.5	3.2	1.3	0.2
0	5	1	3	0.1	5.7	0.7	2.2
6	2	1	0	5.0	2.7	0.9	0.2
7	1	0	1	7.2	1.2	0.1	1.2
3	0	3	3	3.0	1.1	2.5	2.8
0	3	3	3	0.3	2.1	2.2	2.7
7	0	1	0	5.9	1.2	0.7	0.3
4	0	2	3	3.8	1.2	1.1	3.1
1	5	3	0	1.4	5.9	2.2	0.2
6	0	0	2	5.3	1.1	0.0	2.2
5	0	2	2	4.8	1.2	1.2	2.1
5	0	3	1	4.5	1.0	1.7	1.2
4	0	3	2	5.4	1.1	2.6	2.4
2	5	2	0	1.7	6.5	1.5	0.2
3	5	1	0	3.4	3.8	0.5	0.3
4	2	3	0	3.5	1.8	2.2	0.3

Table 5. X-ray Fluorescence Analysis of Individual γ-Alumina Beads Selected from a Pt–Ir–Rh Bead Library after Three Split-Pool Adsorption Cycles

	XRF analysis		
bead number		%	expected composition
1	Pt	0.08	0.1% Pt_1Rh_2
	Ir		
	Rh	0.2	0.2% Pt_1Rh_2
2	Pt	0.23	0.3% Pt_3
	Ir		
	Rh		
3	Pt		
	Ir	0.09	0.1% Ir_1Rh_2
	Rh	0.14	0.2% Ir_1Rh_2
4	Pt	0.05	0.1% Pt_1Ir_2
	Ir	0.19	0.2%
	Rh		

Conclusions

We have demonstrated at the proof-of-concept level that the split-pool method is a viable approach to the synthesis of combinatorial libraries of inorganic materials. The key to success in this method is to ensure uniform, irreversible adsorption of the inorganic components onto chemically and thermally stable support beads. Porous alumina support beads work well for this purpose with acid salts of noble metals and are also attractive because alumina is frequently used as a catalyst support. We have also demonstrated the viability of dye tagging with alumina beads. Dye tagging permits directed sorting between split-pool steps and allows one to control the composition of the library precisely without the statistical redundancy of the random split-pool synthesis. This modification can be particularly important in the design of material libraries containing many components.

Only small libraries of supported transition metal oxides (three components and three split-pool steps) were synthesized in this work because of the length of time needed for XRF analysis of each bead. One can imagine that it should be possible to make much larger libraries by using a larger variety of inorganic precursor salts. For example, a split-pool library made in 8 steps from 10 components would contain over 10^4 unique compositions, according to eq 1. Both dye tagging and XRF appear adequate to differentiate the compositions of beads prepared in eight split-pool steps. In this case, we anticipate that analysis of the bead compositions, rather than library synthesis, will be the bottleneck in combinatorial materials discovery. The micro-XRF technique used here gives reliable bead compositions, but the analysis time is approximately 10 min per bead. Even at this low level of throughput, the synthesis and analysis protocols developed here could be useful if only selected beads (e.g., the most active catalysts) from a large library are analyzed. An alternative strategy would be to combine dye tagging and metal adsorption to minimize the need for XRF analysis. This requires that we find conditions for irreversible metal adsorption that do not involve heating to high temperatures between split-pool cycles. Research along these lines is currently in progress.

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Note Added after ASAP Posting. This manuscript was released ASAP on 8/24/2002 with errors in Table 5 for the expected composition of bead 2 and the Ir analysis result of bead 3. The correct version was posted on 8/28/2002.

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6. The expression "surfactant-silicate" is used here as a comprehensive term for materials synthesized using a mixture of surfactant and silica species, regardless of the particular structure.
7. The transformation between the lamellar and hexagonal mesophases was observed after freeze-drying, as well as air drying, the filtered samples.
8. Addition of trimethylbenzene (TMB) to the reaction mixture stabilizes the lamellar mesophase. Experiments have shown that the 31(±1) Å repeat distance for the layered material shown in Figs. 2A and 3 is preserved over a range of TMB concentrations between 0.5 and 3.0 M, whereas at lower TMB concentrations the hexagonal mesostructure is the favored product. Stabilization of the lamellar mesophase likely occurs because TMB dissolved within the surfactant hydrocarbon assemblies contributes to the hydrophobic chain volume. This increase in surfactant chain volume increases the value of A_o at which the lamellar-to-hexagonal mesophase transformation occurs, according to a simple geometric model (*13*). Thus, the mesostructural transformation depicted in Fig. 2 is a consequence of hydrothermal removal of TMB from within the surfactant chain assembly, combined with an increase in A_o. This conclusion is supported by separate experiments which show that addition of TMB to the aqueous phase inhibits the transformation from a lamellar to a hexagonal mesostructure.
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17. The hexagonal shape of the mesopores can be determined from the y intercept of a plot of d spacings versus the number of carbon atoms for different chain length surfactants, corrected for the head-group diameter.
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23. We thank J. Israelachvili, I. Zisadzinski (UCSB), C. Kresge, D. Olson, J. Beck, J. Vartuli, and J. Higgins (Mobil) for helpful discussions. This research was funded by Air Products, du Pont, the MRL Program of the National Science Foundation under award DMR 9123048, the Office of Naval Research (G.D.S.), the NSF Science and Technology Center for Quantized Electronic Structures (grant DMR 91-20007), the NSF/NYI program, and the Camille and Henry Dreyfus Foundation (B.F.C.) and through fellowships by the FNRS (A.M.) and the DFG (F.S.).

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An Unnatural Biopolymer

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A highly efficient method has been developed for the solid-phase synthesis of an "unnatural biopolymer" consisting of chiral aminocarbonate monomers linked via a carbamate backbone. Oligocarbamates were synthesized from *N*-protected *p*-nitrophenyl carbonate monomers, substituted with a variety of side chains, with greater than 99 percent overall coupling efficiencies per step. A spatially defined library of oligocarbamates was generated by using photochemical methods and screened for binding affinity to a monoclonal antibody. A number of high-affinity ligands were then synthesized and analyzed in solution with respect to their inhibition concentration values, water/octanol partitioning coefficients, and proteolytic stability. These and other unnatural polymers may provide new frameworks for drug development and for testing theories of protein and peptide folding and structure.

Polypeptides have been the focus of considerable attention with respect to their structure and folding, biological function, and therapeutic potential. The development of efficient solid-phase methodology for the synthesis of peptides (*1*), peptide derivatives (*2*), and large peptide libraries (*3–8*) has greatly facilitated these studies. The development of efficient methods for the synthesis of unnatural biopolymers (*9–11*) composed of building blocks other than amino acids may provide new frameworks for generating macromolecules with novel properties. For example, polymers with improved pharmacokinetic properties (such as membrane permeability and biological stability) might facilitate drug discovery, and polymers with altered conformational or hydrogen-bonding properties may provide increased insight into biomolecular structure and folding. We report the highly efficient solid-phase synthesis of oligocarbamate polymers from a pool of chiral aminocarbonates and the synthesis and screening of a library of oligocarbamates for their ability to bind a monoclonal antibody (mAb).

The oligocarbamate backbone (Fig. 1), in contrast to that of peptides, consists of a chiral ethylene backbone linked through relatively rigid carbamate groups. The α carbon, like that of peptides, is substituted with side chains that contain a variety of functional groups. Although the β carbon is unsubstituted in our initial target, additional backbone modifications (and conformational restriction) can be incorporated via alkylation of the β carbon or the carbamyl nitrogen. Oligocarbamates were synthesized from a pool of optically active *N*-protected aminocarbonates (Fig. 2) which, in turn, were derived from the corresponding optically active amino alcohols. The latter are either commercially available or can be prepared in chiral form by reduction of the *N*-hydroxysuccinimidyl or pentafluorophenyl esters of *N*-protected amino acids (*12*). The α-amino group was protected with the use of either nitroveratryl chloroformate (*13*) (NVOC-Cl) (for photochemical deprotection) or fluorenylmethyl-*N*-hydroxysuccinimidyl carbonate (Fmoc-OSu) (for base-catalyzed deprotection) (*14*). When necessary, side chains were protected as acid-labile *tert*-butyl esters, ethers, or carbamates. Protected amino alcohols were converted to the corresponding *N*-protected *p*-nitrophenyl carbonate monomers by reaction with *p*-nitrophenyl chloroformate in pyridine/CH_2Cl_2, generally in >80% yield. The carbonate monomers are stable for months at room temperature.

Solid-phase synthesis of oligocarbamates involves the sequential base-catalyzed or light-dependent deprotection of the α-amino group of the growing polymer chain followed by coupling to the next protected *p*-nitrophenyl carbonate monomer (Fig. 2). The *N*-protected "hydroxy-terminal" residue was covalently attached to polystyrene resin containing either *N*-protected *p*-alkoxybenzyl amino

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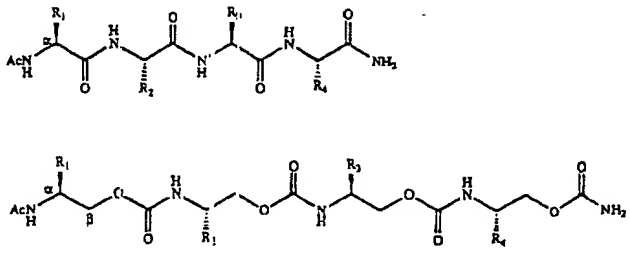
acid ester (*15*) or 4-(2′,4′-dimethoxyphenyl-aminomethyl)-phenol (*16*) linkers. A typical coupling cycle involved: (i) deprotection of the resin Fmoc group by treatment with 20% piperidine in *N*-methylpyrrolidinone (NMP); (ii) washing of deprotected resin with NMP; (iii) coupling with 100 mM Fmoc carbonate monomer in 50 mM diisopropylethylamine (DIEA), 200 mM hydroxybenzotriazole (HOBt) in NMP for 4 hours at 25°C; and (iv) washing of resin with NMP. Side chain deprotection and cleavage from the resin were carried out as described for peptide synthesis (*17*). The products and yields of individual coupling reactions were monitored by analytical reversed-phase high-performance liquid chromatography (HPLC) and quantitative ninhydrin tests (*18*). Overall coupling yields were greater than 99% per step. Oligocarbamates were purified by preparative HPLC and characterized by fast atom bombardment mass spectrometry and nuclear magnetic resonance spectroscopy (*19*).

In order to demonstrate that libraries of oligocarbamates can be generated and screened for receptor binding, an oligocarbamate library was synthesized by using a light-directed parallel synthesis method previously described for the generation of oligopeptide libraries (*4*). This approach permits the spatially addressable synthesis and screening of individual oligocarbamates for receptor binding, thereby obviating the need for sequence analysis. Synthesis was carried out with NVOC-protected monomers that were deprotected by irradiation at 365 nm (*4*). A library containing 256 oligocarbamates was synthesized with a binary masking strategy with the parent sequence $Ac{Y}^{c}F^{c}A^{c}S^{c}K^{c}I^{c}F^{c}L^{c}$ (this library contains all possible deletions of the parent sequence) (*4*, *20*). Carbamate coupling yields on the glass surface were determined as previously described for peptide couplings and were of comparable efficiency (≥90%) (*4*).

The oligocarbamate library was then screened for its ability to bind the α-$Ac{Y}^{c}K^{c}F^{c}L^{c}$ mAb, 20D6.3 (*21*), which was prepared by standard methods from BALB/C mice immunized with the keyhole limpet hemocyanin conjugate of $Ac{Y}^{c}K^{c}F^{c}L$ G-OH (*22*) (G-OH represents a terminal Gly residue) (*21*, *23*). The aminopropyl-derivatized glass surface containing the oligocarbamate library was treated with 20D6.3 in 50 mM tris, 150 mM NaCl buffer, pH 7.4, containing 10% calf serum at room temperature. Antibody-oligocarbamate complexes were then detected by scanning epifluorescent microscopy with a goat α-mouse fluorescein-conjugated secondary antibody (Fig. 3) (*4*). The oligocarbamates $AcK^{c}F^{c}L^{c}G$-OH, $AcF^{c}K^{c}F^{c}L^{c}G$-OH, $AcY^{c}K^{c}F^{c}L^{c}G$-OH, $Ac{A}^{c}K^{c}F^{c}L^{c}G$-OH, and $AcI^{c}F^{c}L^{c}G$-OH, which were among the 10 highest affinity ligands based on fluorescence intensities, were then synthesized on an Applied Biosystems model 431A peptide synthesizer. Competition enzyme-linked immunosorbent assay experiments with $Ac{Y}^{c}K^{c}F^{c}L^{c}G$–bovine serum albumin conjugates revealed that all of these ligands bound to 20D6.3 in solution with IC_{50} values between 60 and 180 nM (*22*). In contrast, specific binding of the ligand $Ac{Y}^{c}I^{c}L^{c}G$-OH, which ranked in the bottom 10% of the library, could not be detected up to ligand concentrations of 100 μM. These results suggest that the dominant epitope of 20D6.3 is -$F^{c}L^{c}$. Consistent with this interpretation, 20D6.3 also binds $Ac{Y}^{c}F^{c}L^{c}G$-OH in solution with an IC_{50} on the order of 160 nM. Surprisingly, the fluorescence signal associated with this ligand ranked in the bottom 30% of the library, suggesting that the conformation of this ligand on the solid support may be different from that in solution (*24*). Nonetheless, the oligocarbamate library allowed us to rapidly determine the epitope of 20D6.3.

Because the oligocarbamate backbone (*25*) differs significantly from that of polypeptides one might expect differences in lipophilicity, hydrogen-bonding properties, stability, and conformational flexibility. Comparison of the water-octanol partition-

Fig. 1. Structures of an oligopeptide and the corresponding oligocarbamate (*20*).



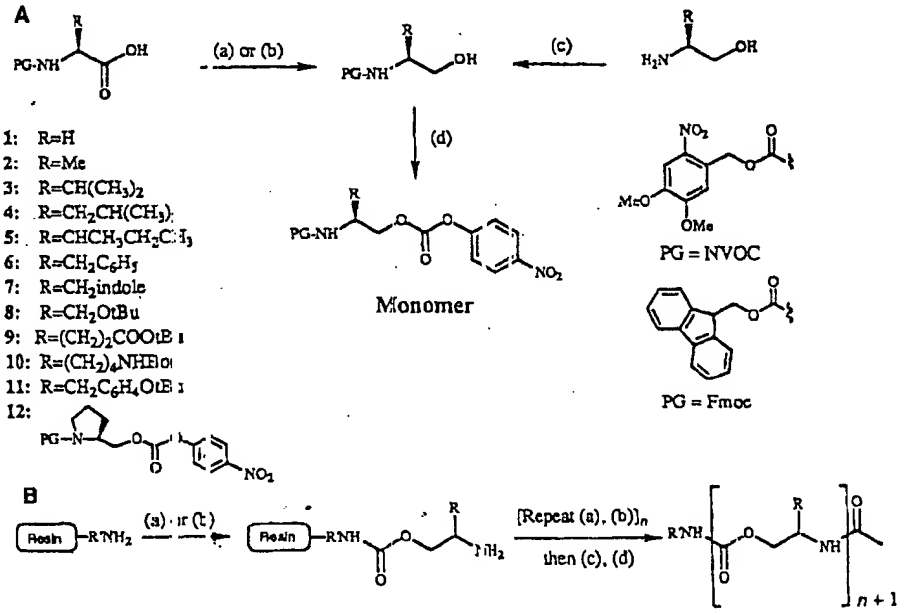


Fig. 2. (**A**) Synthesis of activated *N*-protected *p*-nitrophenyl carbonate monomers: (a) BH_3, tetrahydrofuran; (b) DCC, CH_2Cl_2, *N*-hydroxysuccinimide, HOBt; then sodium borohydride, ethanol; (c) NVOC-Cl or Fmoc-OSu, dioxane, $NaHCO_3$, H_2O; (d) *p*-nitrophenylchloroformate, CH_2Cl_2, pyridine. (**B**) Solid-phase synthesis of oligocarbamates: (a) monomer, HOBt, diisopropylethylamine, NMP; (b) piperidine, NMP; (c) acetic anhydride, NMP; and (d) trifluoroacetic acid, triethylsilane. R = H (Rink resin) (*15*) or amino acid (Wang resin) (*15*)

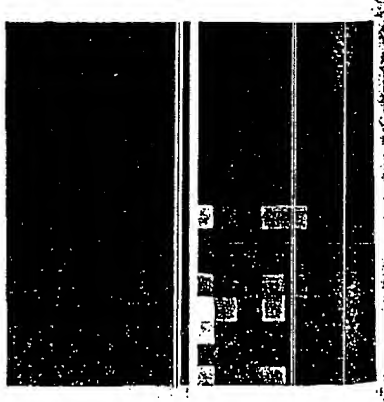


Fig. 3. Fluorescence intensities of oligocarbamate-antibody 20D6.3 complexes detected by using a goat α-mouse fluorescein-conjugated antibody. Each 800 μm by 800 μm square represents an individual member of the library.

ing coefficients (26) of the peptides AcYK-FLG-OH (90) and AcY FLG-OH (10) with the corresponding oligocarbamates $AcY^cK^cF^cL^cG$-OH (0.5) and $AcY^cI^cF^cL^cG$-OH (0.4) revealed the latter to be significantly more hydrophobic. Moreover, oligocarbamates were significantly more resistant to proteolytic degradation than peptides. Treatment of the peptides AcYKFLG-OH and AcYFLG-OH with trypsin or porcine pepsin, respectively, resulted in complete degradation within 20 min whereas the corresponding oligocarbamates showed no appreciable degradation after 150 min (27). These characteristics of oligocarbamates may be reflected in enhanced pharmacokinetic properties relative to oligopeptides. The structural and pharmacological properties of oligocarbamates and other polymers (such as substituted ureas and sulfones) may not only provide new tools for medicinal chemists buy may also provide new opportunities to construct two- and three-dimensional biopolymers with novel properties.

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20. Oligocarbamate sequences are designated by the names of the corresponding amino acids with a superscript c to indicate a carbamate bond. For example the oligocarbamate where R_1 = *p*-hydroxybenzyl, R_2 = 1-aminobutyl, R_3 = benzyl, and R_4 = 2-methylpropyl is designated $Ac\text{-}Y^cK^cF^cL^c\text{-}NH_2$, where Ac is acetyl. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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23. The NHS ester of the nitrophenylsulfenyl-protected hapten was coupled to keyhole limpet hemocyanin, and the resulting conjugate was deprotected by treatment with sodium dithionite to give a hapten to carrier density of 4.
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27. Trypsin (670 nM) was incubated at room temperature with either peptide substrate AcYKFLG-OH (630 μM) or carbamate ligand $Ac\text{-}Y^cK^cF^cL^cG$-OH (420 μM) in tris-saline buffer, pH 7.4, containing 1% DMSO and 1% acetonitrile. Similarly, porcine pepsin (0.1 M formate buffer, pH 3.1, 1.1 μM) was incubated with peptide substrate AcYIFLG-OH (440 μM) or oligocarbamate $Ac\text{-}Y^cI^cF^cL^cG$-OH (390 μM). Degradation of substrates was monitored by reversed-phase analytical HPLC.

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Discovery of Vapor Deposits in the Lunar Regolith

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Lunar soils contain micrometer-sized mineral grains surrounded by thin amorphous rims. Similar features have been produced by exposure of pristine grains to a simulated solar wind, leading to the widespread belief that the amorphous rims result from radiation damage. Electron microscopy studies show, however, that the amorphous rims are compositionally distinct from their hosts and consist largely of vapor-deposited material generated by micrometeorite impacts into the lunar regolith. Vapor deposits slow the lunar erosion rate by solar wind sputtering, influence the optical properties of the lunar regolith, and may account for the presence of sodium and potassium in the lunar atmosphere.

One of the expectations during the Apollo program was that the mineral grains in lunar soils would provide an opportunity to monitor the activity of the ancient sun and the properties of the solar wind as a function of time. However, it was soon realized that determination of the exposure history of individual grains was complicated by regolith processes, namely, meteoroid impact "gardening" on the lunar surface. Nevertheless, some workers tried to deduce the characteristics of the ancient solar wind from data acquired primarily from high-voltage transmission electron microscope (TEM) studies of the fine-grained fractions of lunar soils. It was discovered that many of the mineral grains in the submicrometer size range are surrounded by thin amorphous layers (*1*) that, it was demonstrated, could be produced by exposing mineral grains to a high flux of low-energy ions in the laboratory. This result suggested that the interaction of the solar wind with crystalline grains could produce the thin layer, where the crystallinity of the host grain was destroyed by implantation of solar wind ions (*2*–*4*). The concept that the amorphous rims are a result of radiation damage has been widely cited and is firmly ensconced in the literature. Our examination of the amorphous rims and their host grains shows, however, that most, if not all, of the amorphous rims formed primarily by the

A Class of Cobalt Oxide Magnetoresistance Materials Discovered with Combinatorial Synthesis

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The recent development of methods for generating libraries of solid-state compounds has made it possible to apply combinatorial approaches to the discovery of materials. A library of 128 members containing different compositions and stoichiometries of $Ln_xM_yCoO_\delta$, where Ln = Y or La and M = Pb, Ca, Sr, or Ba, was synthesized by a combination of thin-film deposition and physical masking techniques. Large magnetoresistance has been found in $La_x(Ba,Sr,Ca)_y$-CoO_δ samples, whereas Y-based samples exhibit much smaller magnetoresistive effects. The magnetoresistance of the Co-containing compounds increases as the size of the alkaline earth ion increases, in sharp contrast to Mn-containing compounds, in which the magnetoresistance effect increases as the size of the alkaline earth ion decreases.

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A General Method for Molecular Tagging of Encoded Combinatorial Chemistry Libraries

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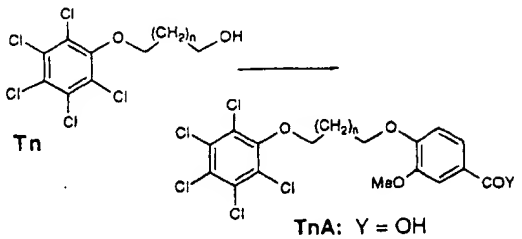
Summary: Acylcarbenes can be used to attach chemically inert, vanillin-linked tagging molecules directly to an unfunctionalized Merrifield polystyrene solid support and thus to allow encoding of combinatorial libraries prepared by a wide range of chemistries.

For complex problems in molecular design, screening massive libraries of diverse molecules prepared by combinatorial chemistry offers a promising alternative to deterministic design. Until recently, such libraries having $>10^4$ members were limited to libraries of oligonucleotides and peptides because direct structure elucidation is generally problematic on the available (picomolar) quantities of individual library members.[1,2] The breakthrough came with *encoding*, a technique that labels each individual library member with a unique array of readily analyzable molecules called *tags*.[3] Each tag array forms a kind of molecular barcode that can be read to determine the structure of associated library members. In all previously described procedures for encoded combinatorial synthesis, tags are added to reactive functional groups (*e.g.*, amines) that are attached either directly or indirectly to the library member.[4,5] However, handling tag-attaching functionality not only complicates library synthesis but can also place undesirable constraints on the reagents and reaction conditions that can be used. In this paper, we describe a new technique for tagging polystyrene-supported combinatorial libraries that requires no particular tag-attaching functional groups other than those (*e.g.*, phenyls) which make up the polymer matrix.

To tag unfunctionalized organic material, we have developed a new type of tagging reagent that is composed of a tag plus a linker bearing a precursor of a reactive intermediate that reacts with a variety of common organic moieties. The tags themselves have been described previously.[5] They are halophenol derivatives (*e.g.*, **Tn**) which are both chemically inert and conveniently analyzed at subpicomolar levels by electron capture gas chromatography (ECGC). The linker is used

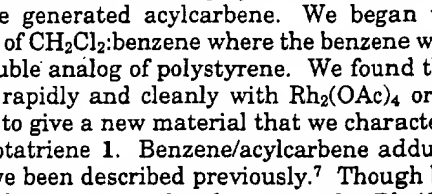


TnA: Y = OH
TnB: Y = Cl
TnC: Y = CHN_2

to attach the tag to the solid support and to facilitate its subsequent detachment for ECGC analysis. In this work, the linker is a derivative of vanillic acid (3-methoxy-4-hydroxybenzoic acid)—the complete tagging reagent is shown above as **TnC**. This construct incorporates diazoketone functionality that can be converted to a reactive acylcarbene for direct attachment to a polymer matrix and a catechol diether moiety that can be cleaved oxidatively to effect tag release for analysis.

Synthesis of the tagging reagent is straightforward. Starting with a tag alcohol (**Tn**), a Mitsunobu reaction is used to attach the linker precursor, methyl vanillate, yielding **TnA** (~60% yield) after saponification (LiOH, MeOH). Oxalyl chloride then gives acid chloride **TnB**, and excess diazomethane yields the tagging reagent **TnC** (~75% from **TnA**). These reagents are stable, yellow solids that can be stored for periods of months at room temperature. Experimental details are provided in the supplementary material.

Our first experiments with tagging reagent **T8C** (**TnC** where $n = 8$) were directed toward finding a suitable catalyst for diazoketone decomposition and a solvent which would both swell the polymer and react only slowly with the generated acylcarbene. We began with 4:1 mixture of CH_2Cl_2:benzene where the benzene was taken as a soluble analog of polystyrene. We found that **T8C** reacted rapidly and cleanly with $Rh_2(OAc)_4$ or $Rh_2(O_2CCF_3)_4$[6] to give a new material that we characterized as cycloheptatriene **1**. Benzene/acylcarbene adducts such as **1** have been described previously.[7] Though both Rh(II) catalysts gave **1** cleanly, we prefer $Rh_2(O_2CCF_3)_4$ because of its high solubility in CH_2Cl_2.



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Our new tagging reagents (**TnC**) are also able to bond directly to polystyrene synthesis beads. However, be-

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(8) Tags appear chemically bound to the solid support particles as judged by comparisons of tag loading before and after 100 washings with CH_2Cl_2 (each washing: 15 min agitation on a wrist action shaker).

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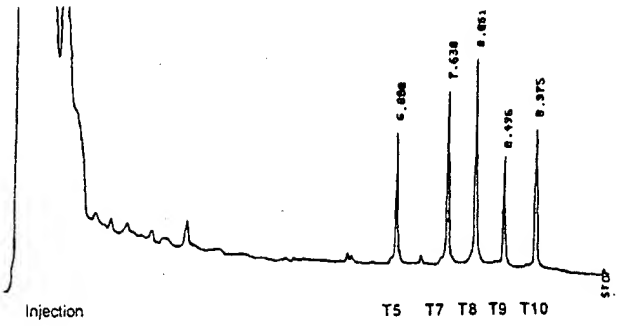
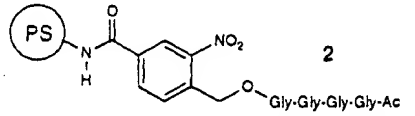


Figure 1. Electron capture gas chromatogram of tags **Tn** from a single synthesis bead.

cause the polystyrene is localized in the form of solid particles, the acylcarbene/arene coupling is less efficient and is accompanied by substantial dimerization yielding a stilbene-like byproduct. Though wasteful of tag, this dimerization is not a serious problem because the soluble dimer is readily removed from the synthesis beads by washing. No other soluble products appear to be formed, and 10–20% of the original **TnC** ends up bonded to the solid support. The best procedure we have found involves thoroughly mixing 50–100 μm Merrifield polystyrene beads with a CH_2Cl_2 solution containing 0.002% of the $Rh_2(O_2CCF_3)_4$ catalyst. Next, a reagent-coding mixture[6] of diazoketone tags (50 mg **TnC** per gram of beads) in CH_2Cl_2 is added with vigorous agitation in three to four equal portions. The diazoketone decomposition and coupling reactions are very fast, provided that any basic groups on the beads (*e.g.*, amines) are either protonated or otherwise protected. This procedure leads to almost uniform tagging of the solid support at a loading corresponding to ~1 pmol of tag per bead.[8]

To read the synthetic information encoded by the vanillin-linked tags, the tags on a particular solid support particle are released oxidatively. Thus, the tags on a single bead are read by first sonicating the bead in a melting point capillary containing 3 μL of hexane and 1 μL of 0.5M ceric ammonium nitrate in 1:1 water:acetonitrile. After centrifugation and removal of the aqueous phase, silylation (*bis*-trimethylsilacetamide) of the released tag alcohols (**Tn**) gives the sample for ECGC analysis. Control experiments with polystyrene supports tagged by an amide bond at the known loading of 0.8 mmol/gm of support using **T8A** indicate that oxidative release occurs in >90% yield. Using the preceding recipes for tag addition and release, we find that tag arrays can be read unambigously from single beads in ≥95% of the cases. For example, Figure 1 shows an ECGC analysis of tags **T5**, **T7**, **T8**, **T9**, and **T10** which were released oxidatively from a single Merrifield synthesis bead (loading ~1 pmol **Tn**/bead).

Because of the extraordinary sensitivity of ECGC of our halogenated tagging molecules, beads need to be tagged at a level (0.5–1 pmol/bead) corresponding to only 0.5–1% of the loading of the library member being synthesized (~100 pmol/bead). Consequently, the tagging procedure does not interfere with the library sythesis, whether the tagging acylcarbene adds to polymer matrix or to the attached library member. Nevertheless, we carried out an experiment to establish the selectivity of our acylcarbenes for attachment to polymer or library member in a simple example. To this end, we prepared the polystyrene-bound, *o*-nitrobenzyl ester-linked tetrapeptide **2**. We tagged this material using reagent **T7C**



2

as described above. By photolysis of the tagged polymer in acetonitrile (λ = 350 nm, 18 h), we were able to release the acetyltetraglycine fragment into solution and to remove the polymer beads. We then treated the tetraglycine fragment solution and the beads separately with ceric ammonium nitrate to release the tag alcohol **T7**. ECGC analysis of these two oxidation products indicated a ratio of tag from the tetraglycine solution to tag from the solid support to be ~1:8. Since our support was functionalized by tetraglycine at a loading of ~0.8 mmol/gm of polystyrene, this ratio corresponds approximately to the relative weights of the polymer matrix and the tetraglycine in **2**. This result suggests that our acylcarbenes add to both polymer and library member with little discrimination. Thus, a typical combinatorial synthesis using 25 tags can be expected to be accompanied by an overall tag-induced destruction of ≤5% of the library member on each bead. Even if the tagging reagents sought out the compound being synthesized and attacked it exclusively, a combinatorial synthesis encoded with 25 tags would still yield 75–85% of tag-free product.

The acylcarbene tagging method described here is a major advance over previous tagging procedures. Not only are no specific functional groups required for tag attachment, but the tags and linkers are generally inert to the often vigorous reaction conditions that are commonly used in organic synthesis. Reagent insensitivity is one of the key advantages of the tagging scheme outlined above, especially in comparison to alternative tagging methods based on biopolymers.[4] Thus, the problem of structure determination in encoded combinatorial libraries is effectively solved by the method described above in a way that is compatible with a wide range of chemistries. The challenge now is to develop solid phase synthetic reactions leading to valuable classes of molecules and screening methods that allow the most interesting compounds to be selected from a combinatorial library.[9]

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Supplementary Material Available: General experimental procedures and characterization data (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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